Type 1 Diabetes Mellitus & Coeliac Disease

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Type 1 Diabetes Mellitus & Coeliac Disease

Clinical aspects and the case for screening

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Thesis: VU University Amsterdam

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VRIJE UNIVERSITEIT

Type 1 Diabetes Mellitus & Coeliac Disease

Clinical aspects and the case for screening

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dr. M. E. Tushuizen

‘Ride hard, fly high and smile up’
Ruben Lenten

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Chapter 1

General introduction and outline of this thesis

General introduction

Coeliac disease (CD) is a permanent intolerance to ingested gluten resulting in immune mediated inflammatory damage to the small intestinal mucosa and a subsequent malabsorption syndrome[1].
CD currently occurs in about 1% of the general population worldwide[2] but differs between the studied populations and used screenings methods. A screening study in the Netherlands revealed that the prevalence of recognized CD was 0.016% and of unrecognized CD 0.35%[3].

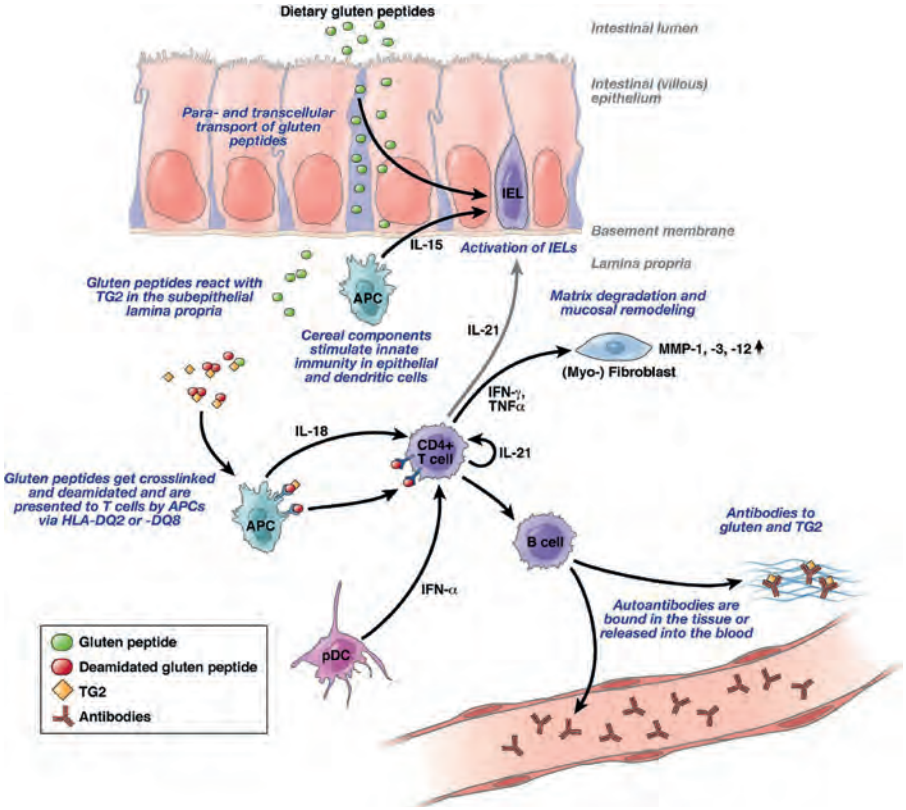
The classic presentation of CD describes symptoms related to gastrointestinal malabsorption and includes malnutrition, chronic diarrhoea, anorexia, constipation, abdominal distension and abdominal pain[4]. However, adult onset patients in particular might also have "non-classic symptoms" such as anaemia, osteoporosis, dermatitis herpetiformis, neurological or psychiatric problems, infertility, aphthous stomatitis, and vitamin deficiencies[5].

Gluten is the trigger of CD and is a protein complex found in wheat, rye, and barley. The pathogenesis of CD involves an external trigger (gluten), changes in intestinal permeability, enzymatically modified gluten, gluten peptide presentation by specific leucocyte antigen (HLA) alleles, and innate and adaptive immune responses to gluten peptides eventually leading to enteropathy (Figure 1)[6;7].
Gluten peptides reach the lamina propria via either epithelial transcytosis or an increased epithelial tight junctional permeability[7]. Deamidation of gluten peptides by Tissue Transglutaminase 2 (TG-2) creates potent immunostimulatory epitopes that are presented via HLA-DQ2 or HLA-DQ8 on antigen-presenting cells. Subsequently, CD4+ T cells are activated, secreting mainly Th1 cytokines such as IFN-γ, which induces the release and activation of MMPs by myofibroblasts, finally resulting in mucosal remodeling and villous atrophy[7]. Additionally, Th2 cytokines are produced driving the production of (auto-) antibodies to gluten and TG2. As gluten is the trigger of CD, the cornerstone of treatment of CD is a glutenfree diet (GFD).

Serologic testing for CD is by antibodies against TG-2 and Endomysium (EMA) which offer both high sensitivity and specificity[8]. Biopsy of the small bowel is necessary to confirm the diagnosis of CD, although recent guidelines from the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) suggest

that a biopsy of the small intestine may not be required in paediatric cases of typical symptoms, a high titre of anti–tissue transglutaminase antibodies and predisposing HLA genotypes[9]. Characteristic histologic changes include an increased number of intraepithelial lymphocytes (>25 per 100 enterocytes), elongation of the crypts, and partial to total villous atrophy[1]. CD is associated with other autoimmune disorders including type 1 diabetes mellitus (T1DM), autoimmune thyroiditis and Addison's disease[10;11].

Figure 1: Pathogenesis of coeliac disease [7].



T1DM is an immune-mediated disorder characterized by a T-cell mediated destruction of the insulin producing β-cells in the pancreas. The subsequent lack of insulin leads to increased blood and urine glucose and may even lead to life-treating diabetic ketoacidosis (DKA)[12]. Long term diabetic complications consist of micro- and macrovascular disease, which account for the major morbidity and mortality associated with T1DM[13]. T1DM precipitates in genetically susceptible individuals,

very likely as a result of an environmental trigger, which is currently unknown[12]. The main diagnostic autoantibodies in T1DM are reactive to four islet autoantigens (islet cell autoantibodies or ICA): insulinoma-associated antigen-2 (I-A2), insulin (mIAA), glutamic acid decarboxylase 65 (GAD65), and zinc transporter 8 (ZnT8)[14].

The mean prevalence of CD in T1DM patients is about 6%[15]. An individual is predisposed for developing CD and T1DM by both HLA and non-HLA genes, in which some gene loci are shared by both disorders[16]. Moreover, gluten exposure and intestinal permeability are thought to be contributing factors in the development of both T1DM and CD[17].

In clinical practice, challenges remain in establishing the diagnosis of CD in T1DM patients. This is in part because of the absence of symptoms in T1DM patients with CD (ranging from 35.7 to 62.5%[18-23]). Some patients who are apparently asymptomatic may have subtle complaints indicative of CD and may only be recognized in retrospect following the benefits of a gluten free diet (GFD)[24]. However, a patient reported delay in diagnosis of CD is frequently found in patients with T1DM[25]. To prevent this delay of diagnosis, screening for CD might be indicated. Screening involves examination of asymptomatic individuals for disease, which is to prevent mortality from the disease under consideration. The World Health Organization (WHO) formulated the Wilson and Jungner criteria to justify screening[26].
Screening for CD in paediatric T1DM patients is advocated. However, international paediatric consensus based guidelines differ in the need and frequency of screening for CD[18]. Some recommend an annual screening interval by testing antibodies against TG-2, others advice to perform these tests in the presence of typical CD symptoms only[18]. Although the prevalence of CD is high and the fact that CD symptoms might be absent, there is no consensus on screening adult T1DM patients for CD. This is stressed by a recent study by Simpson et al which showed that a large variation in screening frequency exists; they showed that the screening frequency in endocrine clinics is 80% while this was 35% in other clinics[27]. The present guideline of the American College of Gastroenterology recommends CD screening in adults with T1DM if there are symptoms suggestive for CD[28]. However, this may lead to missed or delayed diagnosis in this high-risk group, as only 35.7 to 62.5% of these patients report symptoms[18-23]. The consequences of undiagnosed asymptomatic CD in T1DM patients are currently unknown.

Outline of this thesis

Knowledge about the clinical characteristics and long term consequences of patients with both T1DM and CD has grown over the past years, however, several questions remain unanswered. This thesis focuses on the clinical and genetic characteristics of patients with both T1DM and CD. In addition, the need for CD screening in T1DM patients is discussed.

In **chapter 2** we investigated whether common clinical practice in The Netherlands regarding patients with both T1DM and CD is accurate. We performed a retrospective study in which we investigated a large group of patients with both T1DM and CD. We analyzed the age of CD diagnosis and the 'patient reported' delay of CD diagnosis.

The diagnosis and treatment of CD is important to prevent CD related complications in patients with T1DM. CD might also influence the course of T1DM which is scarcely studied. In **chapter 3,** we investigated the effects of CD on T1DM related complications as glucose control, diabetic retinopathy and nephropathy.

The reason for a different prevalence of microvascular complications in T1DM + CD patients versus T1DM patients might be caused by Advanced Glycation End product (AGE's) levels. AGE's play a role in the development of microvascular complications in patients with T1DM and are generated due to oxidative stress and exogenous intake (e.g. heated foods). A recent study found that patients with both T1DM and CD had lower levels of AGE's than patients with T1DM only[29]. This was hypothesized to be related to lower exogenous intake of AGE's because of a GFD. In **chapter 4** we investigated whether AGE's, which are usually low in high-temperature processed foods such as in a GFD, are present in lower levels in patients with both T1DM + CD.

Both T1DM and CD are chronic diseases which affect the quality of life (QOL). Knowledge about the effect of both T1DM and CD on QOL is of importance to improve general wellbeing and for guidance and follow-up of these patients. In **chapter 5** we compared QOL in patients with both T1DM + CD versus patients with T1DM only versus the general population.

Both T1DM and CD are autoimmune diseases with shared genetic origin. Both are associated with the major histocompatibility complex class 2 antigens DQ 2.5 and DQ 8 and also with several non-HLA loci. In **chapter 6** we explored the genetic differences between patients with both T1DM and CD versus those with only CD or T1DM.

Chapter 7 provides a review on the clinical effects of CD in T1DM patients and we discuss the myths, controversies and pitfalls of screening for CD in T1DM patients. Further, we propose an algorithm for clinicians to test for CD in T1DM patients.

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Chapter 2

Frequent delay of coeliac disease diagnosis in symptomatic patients with type 1 diabetes mellitus: clinical and genetic characteristics.

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European Journal of Internal Medicine 2013; 24(5):456-460.

Abstract

Background: Patients with type 1 diabetes mellitus (T1DM) are more prone to develop other auto-immune diseases, including coeliac disease (CD). Paediatric patients with T1DM are screened for CD, whereas in adult T1DM patients screening programs for CD are not standardised. The aim of this study was to investigate clinical and genetic characteristics of patients with both diagnoses so as to lead to better detection of CD in adult patients with T1DM.

Methods: We studied 118 patients with both T1DM and CD identified in the Netherlands. We retrospectively collected data on sex distribution, age of onset of T1DM, age of CD diagnosis, CD complaints, duration of CD complaints before CD diagnosis, family history of CD or T1DM, comorbidity and HLA-DQ type.

Results: Thirty-three percent of T1DM+CD patients reported CD related complaints for at least 5 years before CD diagnosis. Two peaks in the age of CD diagnosis in T1DM patients were observed: around 10 and 45 years of age. Women were diagnosed with CD at a younger age than men (median 25 years (IQR 9-38) versus 39 (12-55) years, respectively, $P< 0.05$).

Conclusions: A delay of CD diagnosis is frequently found in adult T1DM patients and two peaks in the age of CD diagnosis are present in T1DM patients. This observational study emphasises that more frequent screening for CD in particularly adult T1DM patients is required, preferably by a 5 years interval.

Introduction:

The association between type 1 diabetes mellitus (T1DM) and coeliac disease (CD) has been recognised since the early seventies of the previous century[1]. Numerous studies to evaluate the efficacy of screening in these patient populations have been performed[2,3]. The prevalence of CD among T1DM patients is approximately 5% and has been reported to be up to 12.3% in Europe[3,4]. The prevalence of CD in the general population is about 0.6% and depends on geographical region[5]. An individual is predisposed for developing CD and T1DM by both HLA and non-HLA genes, in which some gene loci are shared by both disorders[6,7]. Moreover, gluten exposure and intestinal permeability are thought to be contributing factors in the development of both T1DM and CD[8].

Despite increasing knowledge regarding the high prevalence of CD in T1DM patients, much remains unclear regarding the clinical presentation and characteristics of those patients with both T1DM and CD[2,3]. Current guidelines for the paediatric patient advice to screen by antibodies against tissue transglutaminase (TTG) at the time of T1DM diagnosis and bienially thereafter[9]. However, in the adult situation, screening for CD remains a source of ongoing debate[10-12]. As the incidence of T1DM[13,14] and the prevalence of CD[15] is rising, standardised screening methods are required.

It is well-known that CD has a broad clinical spectrum of presentation with a large proportion of patients presenting without any complaints[16,17]. Patients who are apparently asymptomatic may have subtle complaints indicative of CD and may only be recognised in retrospect following the benefits of a gluten free diet (GFD)[3]. Early detection of CD, however, is important as delay in diagnosis carries the risks of iron deficiency anaemia, osteoporosis, gastrointestinal malignancies and diminishes the quality of life[18,19]. To date, little is known regarding the distribution of age of diagnosis in patients with both diseases and this information might help the clinician in suspecting patients for CD.

As previously stated, screening guidelines for CD in paediatric and adult T1DM patients differ; in adults they are mainly based on case-finding, i.e. screening in case of symptoms suggestive of CD[9,10]. Therefore, we investigated whether the current clinical practice to diagnose CD in paediatric and adult T1DM patients in the Netherlands is adequate and evaluated clinical and genetic characteristics of patients diagnosed with both T1DM and CD.

Materials and Methods:

Patients

Between September 2010 and September 2012, internists, gastroenterologists, and paediatricians of local and academic hospitals in the Netherlands were asked to identify patients who have been diagnosed with both T1DM and CD. Furthermore, we requested patients with T1DM and concomitant CD to participate in this observational study by advertisement in journals of the Dutch Coeliac Disease Society and the Dutch Diabetes Society.

After informed consent, all patients were interviewed by a single investigator (SFB) and data concerning age of onset of T1DM, age of diagnosis of CD, complaints at diagnosis of CD, duration of CD complaints before diagnosis, date of start of GFD, difficulties of following a GFD, (autoimmune) comorbidity (e.g. autoimmune thyroiditis, Addison's disease) and family history of CD or T1DM in first degree relatives, were obtained.

The mode of CD presentation was defined as the main symptom at the moment of CD diagnosis. There were 5 major modes of presentation: gastrointestinal complaints, tiredness due to anaemia, hypoglycaemia with fluctuating glucose levels, no complaints and others (e.g. dermatitis herpetiformis, growth problems). Delay in diagnosis of CD was determined by asking for the duration of those complaints before CD diagnosis was made. Delay of CD diagnosis was categorized into less than 6 months, between 6 and 12 months, between 1 and 5 years and more than 5 years. Patients without any reported complaints were excluded from this specific analysis.

We retrospectively evaluated the diagnosis of T1DM and CD in all included patients. T1DM diagnosis was defined according to the American Diabetes Association position statement[10]. In brief, T1DM diagnosis was based on T1DM related clinical characteristics together with an absolute requirement of insulin or the presence of antibodies against glutamic acid decarboxylase (GAD). Diagnosis of CD was retrospectively re-evaluated and diagnosed according to the modified European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines[12]. That is, the presence of (sub-total) villous atrophy by intestinal biopsy and the presence of antibodies against tissue transglutaminase (TTG) or endomysium (EMA).

To assess the clinical practice regarding CD screening in T1DM patients, our patient cohort was divided in patients with CD diagnosis before (group A) and after (group B) the age of 18 years. The local ethics committee of the VU University Medical Centre approved the study and the investigation conformed to the principles outlined in the Declaration of Helsinki.

Biochemical measurements

Whole blood was obtained for typing of HLA-DQA1* and DQB1* alleles, performed with a combined single stranded conformation polymorphism (SSCP/heteroduplex method by a semi-automated electrophoresis and gel staining method) PCR on the Phastsystem™ (Amersham-Pharmacia-Biotech, Sweden)[20]. Alleles DQA1*05 and DQB1*02 (encoding the HLA-DQ2.5 dimer) and alleles DQA1*03 and DQB1*0302 (encoding the HLA-DQ8 heterodimer) could be reliably characterised in homozygous and heterozygous states.

Statistical analysis:

Data are shown as medians with interquartile ranges (IQR). The Mann-Whitney U test was used for unpaired data. The Fisher's exact test was used for comparison of categorical data. A P-value of less than 0.05 was considered significant. Statistical analysis was performed using Statistical Package for the Social Sciences (IBM, version 20.0).

Results:

Patient characteristics

We studied data from 118 patients with both T1DM and CD treated in several hospitals in the Netherlands of whom 64% were women (Table 1). In this retrospective cross sectional study, the median age of the total group was 36 years (IQR 17-53). The majority of the patients were diagnosed with T1DM first and then with CD, whereas a small subset of patients (8.5%) was diagnosed with CD first and with T1DM secondly (Fig. 1). A bimodal distribution of the age of diagnosis of CD in T1DM patients was observed with a peak incidence around 10 and 45 years of age (Fig. 2). Forty-two percent of T1DM patients was diagnosed with CD 10 years after T1DM onset.

Women were diagnosed with CD at a younger age than men (25 years (IQR 9-38) versus 39 (12-55) years, respectively, $P< 0.05$). No difference in the duration of CD

related complaints was observed between women and men (P=0.82). Age of onset of T1DM was equal between women and men (11 years (IQR 5-23) versus 11 years (IQR 5-22), P=0.38). Patients diagnosed with CD first and T1DM secondly (N=10) developed T1DM at a significantly older age than the other patients (20 years (IQR 8-43) versus 9 years (IQR 5-21), P<0.05).

Clinical symptoms of CD

A large proportion of patients reported gastrointestinal complaints (45%). No differences were observed in CD related complaints between men and women (P=0.30). To study differences in CD diagnosis in paediatric and adult T1DM patients, we compared patients with CD diagnosis before 18 years (group A) with those with CD diagnosis at adult age (group B), see Table 1. Of the patients in group B, 48% reported CD related complaints for at least 5 years (Table 1). In contrast, the majority (59%) of the patients diagnosed with CD in childhood reported a duration of CD related complaints for less than 6 months. Overall, 33% of the patients reported CD related complaints for a duration of longer than 5 years before CD diagnosis. Twenty-eight percent of all patients did not report to have had any CD related complaints (N=33) and were found by screening (Table 1). Of these patients, 55% was diagnosed with CD before 18 years of age and 45% thereafter (P=0.14).

The calendar year in which CD diagnosis (1969-2012) was made in those T1DM patients was not related to the duration of CD related complaints (P=0.78). Between the patients who were diagnosed first with CD (n=10) or T1DM (n=108) no differences in type of complaints or delay of CD diagnosis were observed (P=0.86 and P=0.95).

Concomitant presence of other autoimmune diseases

In 28% of our patients with both T1DM and CD other accompanying autoimmune diseases were present (Table 1). Autoimmune thyroiditis was the most prevalent concomitant autoimmune disease, occurring in 22% of the patients. Furthermore, systemic lupus erythomatosous, vitiligo, rheumatoid arthritis, ulcerative colitis and sarcoidosis were among the other concomitant diagnoses.

No difference was observed in the presence of other autoimmune diseases between men and women in our population (21% versus 27%, respectively, *P*=0.66). No difference in HLA-DQ type was observed between patients with

another autoimmune disease or without (P=0.43). Between the patients who were diagnosed first with CD or T1DM, no differences in prevalence of another autoimmune disease was observed (P=0.24). In the total group at least one first degree relative was diagnosed with CD in 14% and with T1DM in 23% of the patients.

Table 1: Comparative data of 118 type 1 diabetes mellitus (T1DM) patients with coeliac disease (CD). Groups are divided according to age of diagnosis of CD: before 18 years of age (group A), or after 18 years of age (group B).

	Group A n (%)	Group B n (%)	*P* Value	Total n (%)
Total number of patients	47 (40)	71 (60)		118 (100)
Women	33 (70)	42 (59)	0.22	75 (64)
Age (years)	14 [10-19]	47 [39-60]	< 0.05	36 [17-53]
Age at T1DM diagnosis (years)	7 [3-11]	41 [32-53]	< 0.05	11 [5-22]
Age at CD diagnosis (years)	8 [4-12]	44 [33-59]	< 0.05	26 [10-43]
Other autoimmune diseases	10 (21.3)	23 (32.4)	0.19	33 (28)
Autoimmune thyroiditis	10 (21.3)	16 (22.5)		26 (22)
Others	0	7 (10.9)		7 (6)
First degree relative with T1DM	9 (19.1)	18 (25.4)	0.43	27 (23)
First degree relative with CD	4 (8.5)	12 (16.9)	0.19	16 (14)
Complaints at CD diagnosis			0.07	
Gastrointestinal complaints	20 (42.6)	33 (46.5)		53 (44.9)
Anaemia	5 (10.6)	18 (25.4)		23 (19.5)
Hypoglycaemia	2 (4.3)	4 (5.6)		6 (5.1)
No complaints	18 (38.3)	15 (21.1)		33 (28)
Others	2 (4.3)	1 (1.4)		3 (2.5)
Duration of CD complaints*			<0.05	
< 6 months	13 (59.0)	9 (18)		22 (30.6)
6-12 months	3 (13.6)	8 (16)		11 (15.3)
1-5 years	6 (27.3)	9 (18)		15 (20.8)
> 5years	0	24 (48)		24 (33.3)
HLA-DQ type			0.61	
HLA-DQ2.5/X	9 (25)	17 (30.4)		26 (28.3)
HLA-DQ2.5 homozygous	9 (25)	19 (33.9)		28 (30.4)
HLA-DQ8 and HLA-DQ2.5	8 (22.2)	11 (19.6)		19 (20.7)
HLA-DQ8/X	7 (19.4)	6 (10.7)		13 (14.1)
HLA-DQ8 homozygous	3 (8.3)	2 (3.6)		5 (5.4)
No-HLA DQ2.5 or DQ-8	0	1 (1.8)		1 (1.1)

Data are medians, [interquartile range]. P-value indicates the differences between group A and B
*= Patients found by screening are excluded in the duration of CD related complaints
X= Any except HLA-DQ2.5 or HLA-DQ8

HLA-DQ type

HLA-DQ typing was performed in 78% (N=92) of the T1DM + CD patients. HLA-DQ2.5 heterozygosity was prevalent in 79% of the patients, of which 30% were HLA-DQ2.5 homozygous and 21% HLA-DQ2.5/ DQ8 heterozygous (Table 1). One T1DM + CD patient was negative for HLA-DQ2.5 or HLA-DQ8 and this

patient is carrier of HLA-DQ 2.2. No differences in HLA-DQ type were observed between the patients who were first diagnosed with CD or T1DM (P=0.56).

Figure 1: Scatterplot of the age of diagnosis of coeliac disease on the X-axis and the age of diagnosis of type 1 diabetes mellitus on the Y-axis.

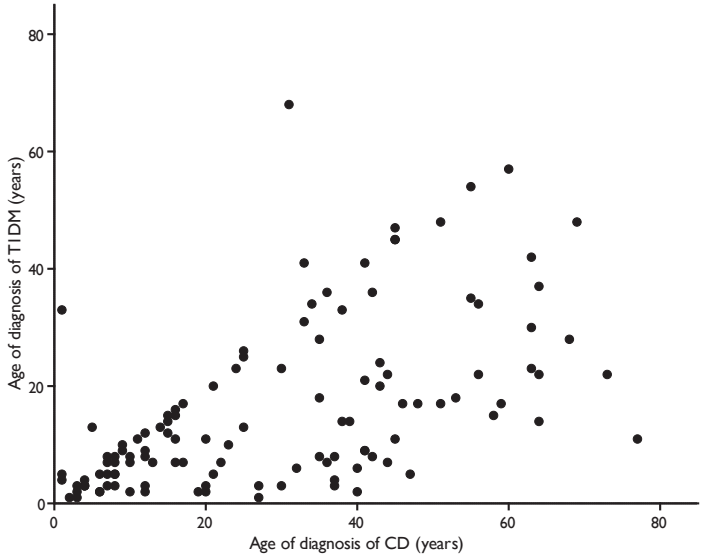
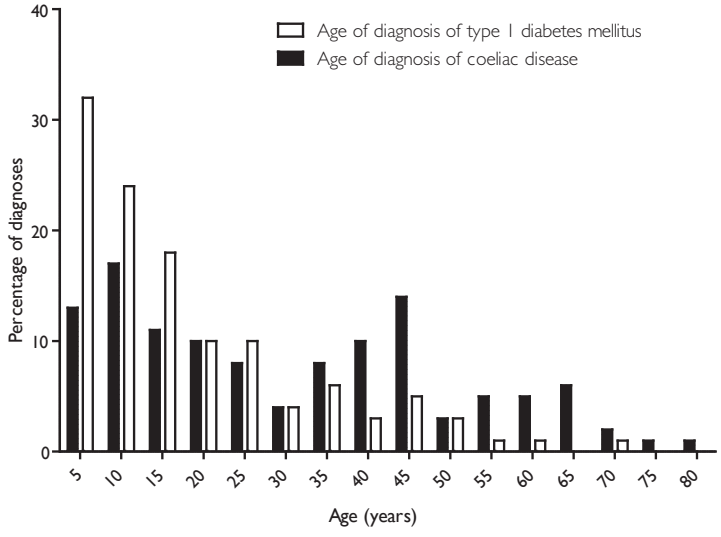


Figure 2: Graph of the age of diagnosis of coeliac disease and of type 1 diabetes mellitus. Age of onset of type 1 diabetes mellitus is indicated in white bars and age of diagnosis of coeliac disease is indicated in black bars.



Discussion:

In the present study, a large proportion (48%) of our patients diagnosed with CD in adulthood reported to have had CD related complaints over 5 years before CD diagnosis was established. Furthermore, we observed a bimodal distribution of the age of diagnosis of CD in T1DM patients with a peak incidence at the age of 10 and 45 years. Women are more prone to have both T1DM and CD and in women CD diagnosis was made at a younger age.

Studies in the general population have shown that CD is often diagnosed after several years of clinical symptoms[21-23]. In our T1DM population we found that a large part had reported CD related complaints for more than 5 years before diagnosis. Although we did not assess the rate of misdiagnosis of CD in our T1DM patients, previous studies reported that in a large part of CD patients the diagnosis of Irritable Bowel Syndrome was made prior to the correct diagnosis of CD[24,25]. Here physician's delay might play a role as T1DM patients are seen annually at the out-patient clinics. Testing IBS patients for undetected coeliac disease using serology has been shown to be cost-effective[26] and is clearly necessary in the individual patient with T1DM. Our observed bimodal distribution of age of onset of CD in T1DM patients has been noted before in CD patients in the general population in the USA[23]. In their study the majority of CD patients were diagnosed with CD in their fourth to sixth decades which is similar to our findings in T1DM patients.

In general, the prevalence of autoimmune diseases is higher in women than in men and the finding of our survey that 64% of the patients with both diseases were women is consistent with other reports. It suggests that sex-linked genetic susceptibility might be involved in the aetiopathogenesis of these diseases[27,28] . The rationale that women are younger at CD diagnosis is as yet elusive, a different clinical presentation or shorter duration of CD related complaints was not observed in our study. So far, studies investigating patients with both T1DM and CD revealed that those patients have a younger onset of T1DM[27,29]. These findings suggest more frequent screening for CD in those T1DM patients with early onset.

Remarkably, only 8.5% of the patients with both T1DM and CD were diagnosed with CD before T1DM onset. In a large multicentre study in Italy, the same pattern was found[27]. This might partly relate to differences in clinical presentation of both

diseases. In contrast to CD diagnosis a delay in T1DM diagnosis is not expected because of the often severe presentation (e.g. diabetic ketoacidosis). Surprisingly, Valerio et al found that patients from this small subgroup presented with a more severe clinical T1DM presentation and higher prevalence of multiple autoimmune diseases[28]. Although we observed a high prevalence (28%) of at least a third autoimmune disease in our T1DM + CD patients, this was not significantly increased in the patients in which CD diagnosis precedes T1DM diagnosis (50%). As our study population is larger and has a longer disease duration, the previous reported findings might be caused by random variation.

We observed comparable data in our T1DM + CD patients regarding the prevalence of T1DM or CD in first degree relatives. The prevalence of 16% of CD in first degree relatives is in accordance with the literature in which this is reported to be up to 20%[30]. This underscores the need for clinicians for awareness for the presence of autoimmune diseases in such families.

The main determinant of risk of developing CD is the HLA DQB1*02- DQA1*05 (HLA-DQ 2.5) haplotype and for T1DM both the HLA DRB1*03-DQB1*0201 and DRB1*04-DQB1*0302 (DR3-DQ2.5 and DR4-DQ8) haplotype[31,32] . Our data confirm the high prevalence of HLA-DQ 2.5 haplotypes in patients with both T1DM and CD[7,33]. The high prevalence of HLA-DQ2.5 homozygosity (30%) in our T1DM + CD population suggests that this confers the highest risk for T1DM patients to develop CD, as was reported before by Sumnik et al[7]. However, HLA-DQ typing could only be helpful in excluding the possibility of developing CD in the future.

The frequent visits of T1DM patients to their physicians on a regular basis suggests that physicians should be more aware of the symptoms and/or the association of both diseases. Therefore, there is a need for a CD screening protocol in adult patients with T1DM (and other associated diseases), which is currently lacking in international guidelines[10]. The finding that 59% of the patients diagnosed with CD in childhood reported complaints for less than 6 months emphasises that CD diagnosis in children is more adequate than in adult T1DM patients. A screening algorithm for CD in adult T1DM patients was proposed recently by Volta et al[34]. They suggest to screen at T1DM diagnosis, once a year for the following 4 years and every 2 years for the following 6 years. Our data suggests to continue the screening for CD after 10 years of T1DM diagnosis. We show that a large proportion (42%) of the T1DM

patients is diagnosed with CD 10 years after T1DM onset and that delay in CD diagnosis is often present.
Limitations that apply to our study are foremost, that our patient population was already diagnosed with CD and this is not a screening study, therefore selection bias is likely. However, our study does represent the current clinical practice in diagnosing CD in T1DM patients in the Netherlands. The distribution of age of diagnosis of CD is highly variable and is dependent on the physicians awareness for CD. Therefore, the bimodal distribution might not be the exact distribution as this is not a screening study by antibodies against tissue transglutaminase or endomysium. Another limitation of our study is recall bias. The elapsed time between start of CD related complaints and the moment of CD diagnosis might be overestimated.

In summary, we here show that a delay in CD diagnosis is frequently found and that a bimodal distribution of CD diagnosis in T1DM patients exists. We suggest to screen for CD in T1DM patients, even after 10 years of T1DM diagnosis, with a regular interval, for instance every 5 years. This is supported by the knowledge that early CD diagnosis and treatment by a GFD protects bone mineral density, prevents iron deficiency, improves quality of life and plays a role in the prevention of developing gastrointestinal malignancy[18]. Furthermore, our observations that a quarter of our patients did not report any CD related complaints, emphasise the need for more frequent CD screening, especially in adults.

Learning points

- Whether screening for coeliac disease (CD) in adult patients with type 1 diabetes (T1DM) should be standardized is matter of debate. We found that a large proportion (48%) of patients diagnosed with CD in adulthood reported to have had CD related complaints over 5 years before CD diagnosis was established.
- A bimodal distribution was observed in the age of diagnosis of CD in T1DM patients, with peaks around the age of 10 and 45 years.
- More frequent and standardised screening in symptomatic T1DM patients, especially in adults, is necessary for the early detection of CD to prevent delay in CD diagnosis and CD related complications (iron deficiency anaemia, osteoporosis).

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Chapter 3

Type 1 diabetes and coeliac disease in adults: glycaemic control and diabetic complications

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Abstract

Background: The prevalence of Coeliac Disease (CD) in patients with Type 1 Diabetes Mellitus (T1DM) is 4.5%. Objective of the study is to investigate 1) the course of glycaemic control at CD diagnosis and after initiation of a gluten free diet (GFD) in T1DM patients; 2) the prevalence of diabetic complications in T1DM patients with adult onset of CD.

Methods: In 20 hospitals in the Netherlands we identified T1DM patients diagnosed with CD at adult age. We retrospectively collected glycated hemoglobin (HbA1c) levels before CD diagnosis, at CD diagnosis and the most recent HbA1c levels as well as the presence of nephropathy and retinopathy. The control group consisted of patients with T1DM and negative CD serology matched for age, gender, T1DM duration and HbA1c levels.

Results: Thirty-one patients were eligible with a median duration of T1DM and CD of 27 years (IQR 14-37) and 3 years (IQR 1-8), respectively. The matched control group consisted of 46 patients. HbA1c levels at the moment of CD diagnosis were 7.5% (IQR 7.1-8) [58 mmol/mol] and at the most recent visit 7.4% (IQR 6.9-7.9, P=0.15) [57 mmol/mol] indicating no difference. Prevalence of retinopathy was lower in T1DM + CD group compared with controls, (38.7% versus 67.4%, P<0.05), whereas no difference in the prevalence of nephropathy was found between the groups (P=0.09).

Conclusions: In conclusion, T1DM+CD patients have less retinopathy compared to T1DM patients without CD. A GFD possibly favourable affects development of vascular complications in T1DM patients.

Introduction:

Type 1 diabetes (T1DM) is an autoimmune disease characterized by T-cell mediated destruction of the insulin-producing islets ß-cells[1]. Patients with T1DM are at increased risk of developing micro- and macrovascular complications and cardiovascular disease compared with the general population[2]. Intensive insulin therapy to regulate blood glucose levels in the normal range effectively delays the onset of and slows the progression of microvascular complications including diabetic retinopathy, nephropathy and neuropathy in T1DM patients[3-5]. Long term follow-up revealed that risk reduction for cardiovascular disease and mortality was associated with the initial HbA1c reduction[6].

Due to a common genetic background and interplay between environmental and immunological factors, patients with T1DM have an increased risk of developing other autoimmune disorders. The most frequent autoimmune disorders diagnosed in T1DM patients are autoimmune thyreoiditis and coeliac disease (CD)[7]. CD is a permanent intolerance to ingested gluten that results in immunologically mediated inflammatory damage to the small intestinal mucosa and therefore leads to a malabsorption syndrome[8]. CD is one of the commonest lifelong disorders encountered in Western countries with a prevalence of about 0.6 % in the general population[9]. The association between T1DM and CD is a well defined fact with an estimated prevalence of CD in T1DM patients of 4.5 %[10].

Data are sparse concerning the effect of concomitant CD in T1DM patients on glycaemic control. Patients with T1DM usually follow a low-caloric diet to control their metabolic state and this might not be easy to maintain together with a gluten free diet (GFD). This GFD could possibly influence glycaemic control. Interestingly, studies addressing this effect are conflicting in adult T1DM patients[11,12]. However, this could be of utmost importance since a tight regulated glycaemic control is essential to prevent development of diabetic retino-, neuro-, and nephropathy[2,13].

Recently, studies have attempted to address the question whether concomitant CD is of influence on the long term diabetic complications. The role of CD on the development of diabetic long term complications has been reported either to be protective or to be aggravating[14-16]. Addressing the question whether CD influences long term complications might help to answer the question whether screening

for CD in adult T1DM patients is necessary. In addition, debate exists regarding the timing and frequency of screening for CD in T1DM patients[17]. In paediatric patients with T1DM, Consensus Based Guidelines differ in the need and frequency of screening for CD. Screening intervals by tissue transglutaminase (TTG) antibodies vary between every year since T1DM diagnosis and 'screening' only in the presence of typical CD symptoms[17]. In the adult situation, however, evidence for specific screening intervals is lacking, whereas anti-TTG testing is mainly indicated in case of clinical CD presentation.

Therefore, the aim of the present study is to further clarify the effect of adult onset of CD on the clinical course of T1DM patients. We identified patients with T1DM and concomitant CD and performed a retrospective study in the Netherlands. Objective of the present study is to investigate: 1) the course of glycaemic control at CD diagnosis and after initiation of a GFD in T1DM patients; 2) the prevalence of diabetic complications in T1DM patients with adult onset of CD.

Materials and methods:

Patients and controls

Between September 2010 and October 2011, physicians of 20 local and academic hospitals in the Netherlands were asked to identify adult T1DM patients who were diagnosed with CD at adult age (> 20 years). Secondly, by advertisement in the journals of the Dutch Coeliac Disease Society and the Dutch Diabetes Society, patients with T1DM and concomitant CD were asked to participate in this observational study. Ethical approval was sought and approved according to the local protocol of the Ethical committee of the VU University Medical Centre.

After CD diagnosis all patients were advised to start with a GFD excluding foods containing wheat, rye, barley and oats by their treating physician. All patients were interviewed by a single investigator (SB) about complaints at diagnosis of CD, date of start of GFD, change of CD complaints after start of a GFD, difficulties of following a GFD, comorbidity, complaints suggestive of retinopathy, neuropathy or nephropathy and family history of CD or T1DM.

A retrospective chart audit in the hospitals of the recruited patients was performed to confirm the diagnosis of T1DM and CD. T1DM diagnosis was according to the American Diabetes Association position statement[18]. The diagnosis of CD was

retrospectively re-evaluated and diagnosed according to the modified European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) criteria[19].

Additional data found by a retrospective chart audit were gastrointestinal complaints, comorbidity, medication use, presence of microalbuminuria, ophthalmology notes, insulin dose (U/day) and anthropometric measurements (height, weight, blood pressure). Further, laboratory results were retrieved from the case files and were glycated haemoglobin levels (HbA1c levels (%)), total cholesterol (mmol/l), High Density Lipoprotein (HDL) cholesterol (mmol/l), Low Density Lipoprotein (LDL) cholesterol (mmol/l) and triglycerides (mmol/l). Body Mass Index (BMI) was defined as the individual's body weight divided by the square of his or her weight.

The participants of the control group were recruited from one local hospital and included T1DM patients that were tested negative for CD, after informed consent. To investigate the effect of treatment for CD, that is GFD, the control group was matched for age, gender, duration of T1DM and HbA1c.

Biochemical measurements

Glycaemic control was investigated by HbA1c levels. Retrospectively, we collected HbA1c levels before the moment of CD diagnosis, at the moment of CD diagnosis and at the most recent HbA1c values. In the study group we analyzed the HbA1c course intra individually. HbA1c was measured with cation exchange chromatography (Menarini Diagnostics, Florence, Italy: reference values; 4.3-6.1%). Total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were detected by enzymatic methods (Modular, Hitachi, Japan).

Long term diabetic complications

All complications were found in the patient charts with a maximum duration of these history notes of 6 months. Retinopathy was graded using the International Clinical Diabetic retinopathy Disease Severity Scale[20]. Retinopathy was defined as the presence of any of the following lesions: microaneurysms, retinal hemorrhages hard or soft exudates or intra-retinal microvascular abnormalities. If an individual had diabetic retinopathy graded as questionable or photos were missing, this was reported as unable to grade. Nephropathy was assessed as the presence or history of an increased microalbumin/ creatinine ratio (cut off value of 2.5 mg/mmol in male and 3.5 mg/mmol in female) in an urine portion which was yearly checked by the treating physician.

Statistical analysis

Continuous data is expressed as median and the interquartile range (IQR). Mean differences of continuous variables between the study cohort and the reference control group were assessed using the Mann Whitney UT-test. For dichotomous variables, Fisher's exact test was used. The T1DM + CD patients were compared with matched control population to ensure there were no differences on the basis of those characteristics. A Wilcoxon signed rank-test was used to assess intra-individual change in HbA1c levels. A two tailed probability of $P < 0.05$ was considered as statistically significant. Statistical analysis was performed using Statistical Package for the Social Sciences (version 13.0).

Results:

Demographic data

The studied population included 31 adult T1DM patients with concomitant CD with a median age of 47 years (IQR 41-57) of whom 42.1% were male (table 1). The median age of onset of T1DM was 21 years (IQR 7-36) with a median duration of 27 years (14-37). Of the study population, 45.2% was diagnosed with T1DM before the age of 20 years and 96.8% of the patients were diagnosed first with T1DM followed by diagnosis of CD. Total patient years of CD were 215 years with a median duration for each patient of CD of 3 years (range 1-40). Although no difference was found in the age of onset of T1DM in males and females, i.e. 21 years (10-46) and 22 years (7-25), respectively, females were significantly at a younger age diagnosed with CD than males, 36 years (30-44) versus 48 years (39.5-60.5), respectively (P=0.01).

Glycaemic control in active and treated CD

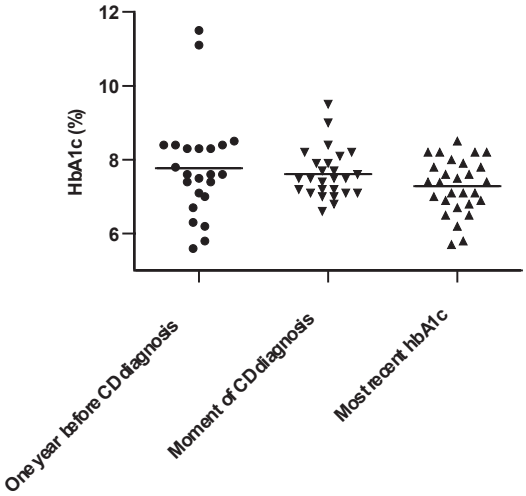
Intra-individual analysis revealed no difference in HbA1c levels between the moment of CD diagnosis and the most recent visit (median 7.5% [58 mmol/mol] (IQR 7.1-8) versus 7.4% [57 mmol/mol] (6.9-7.9),respectively, P=0.15) (Figure 1). The HbA1c levels before CD diagnosis were 7.6% (IQR 7-8.4) and did not differ from HbA1c levels at the moment of CD diagnosis (P=0.88).

Table 1: Comparison of demographic characteristics of adults with T1DM and with CD treated with a GFD and matched control subjects with T1DM. Data are presented as median with IQR.

	Celiac cases (n=31)	Control subjects (n=46)	P-Value
Gender (male)	42.1 %	57.9 %	0.74#
Age in years	47 (41-57)	48 (35-61)	0.77*
Duration of DM in years	27 (14-37)	26 (15-34)	0.71*
Age at diagnosis of T1DM	21 (7-36)	21 (11-28)	0.97*
Insulin dose (u/kg/ day)	0.64 (0.52-0.75)	0.66 (0.54-0.86)	0.21*
Active smoker and/or smoking history (%)	23.1%	43.3%	0.11*
Concurrent autoimmune diseases (%)	26.7%	24.4%	0.61#
Indication for CD screening			
Gastrointestinal complaints	54.9%		
Anemia (iron deficiency)	22.5%		
Hypoglycemia	16.1%		
No complaints	6.5%		
Duration of CD in years	3 (1-8)		
Age CD in years	42 (36-51)		
Body Mass Index	24 (22-29)	25 (21-27)	0.88*
Blood pressure mm/hg			
Systolic	125 (120-145)	126 (117-136)	0.91*
Diastolic	80 (70-83.5)	74.5 (70-80)	0.40*
Blood pressure lowering drugs			
ACE-inhibitors	40%	21.7%	0.14#
Diuretics	3.3%	13%	0.10#
Calcium-antagonist	6.7%	2.2%	0.33#
Total cholesterol mmol/l	4.2 (3.7-4.5)	4.6 (4-5.2)	0.06 #
HDL mmol/l	1.3 (1-1.9)	1.5 (1.2-1.9)	0.16#
LDL mmol/l	2.3 (1.9-2.7)	2.6 (2.2-3)	0.08#
Triglycerides mmol/l	0.8 (0.6-0.9)	0.9 (0.6-1.3)	0.44#
Statin use	29%	28.3%	0.94*
HbA1c (%)	7.4 (6.9 -7.9)	7.5 (7.1-8.4)	0.15*
HbA1c (mmol/mol)	57 (52-63)	58 (54-68)	0.15*

Fisher's exact test used
* Mann-Whitney U-test used

Figure 1: Changes in HbA1c (%) levels before CD diagnosis, at CD diagnosis and at the most recent visit in patients with T1DM and diagnosed at adult age with CD. Time before CD diagnosis has a median of 12 months (IQR10-14).



Cases compared with control subjects

No differences were observed in age of T1DM onset and duration of T1DM, indicating successful matching. Most of the patients diagnosed with CD reported to have gastrointestinal complaints (54.9%) and only a minority (6.5%) of those patients did not report any complaints (table 1). These CD patients without complaints were found by coincidental screening by their treating physician. Hypoglycaemic events before CD diagnosis was reported by 16.1% of the patients. There was no difference in insulin dosage per kg per day between the T1DM+CD and the control group (0.64 vs 0.66 u/kg/day, respectively, P=0.21).

The presence of concurrent autoimmune diseases did not significantly differ between both groups. In the T1DM + CD group, 8 patients had concurrent autoimmune diseases, including hypothyroidism (7 cases) and systemic lupus erythematous. In the control group, however, the concurrent autoimmune diseases were hypothyroidism (n=8), systemic lupus erythematous (n=1), autoimmune gastritis (n=1), psoriasis (n=1), vitiligo (n=1) and polymyalgia rheumatica (n=1).

The prevalence of the long term diabetic complications revealed a significant lower prevalence of retinopathy in the T1DM + CD group compared with matched controls (38.7% versus 67.4%, $P<0.05$), whereas no differences in the prevalence of increased microalbumin/creatinin ratios were found ($P=0.09$) (Figure 2). The distribution of diabetic retinopathy in graded photos is shown in table 2. In the T1DM + CD group gender was equally divided in the group with and without retinopathy ($P=0.62$). There was no difference in systolic blood pressure (128.7 versus 128.0 mm/hg, $P=0.72$) and smoking status (34.5% versus 32.0%, $P=0.98$) between patients with and without retinopathy. In addition, a trend of lower total cholesterol levels was found in the T1DM + CD group (4.2 versus 4.6 mmol/l, $P=0.06$) and lower LDL-cholesterol (2.3 versus 2.6 mmol/l, $P=0.08$) without differences in the use of statins ($P=0.94$). No differences were observed in the levels of HDL-cholesterol or triglycerides.

Figure 2: Comparison of the prevalence of nephropathy and retinopathy in patients with T1DM and the patients with T1DM and CD. NS = Non significant

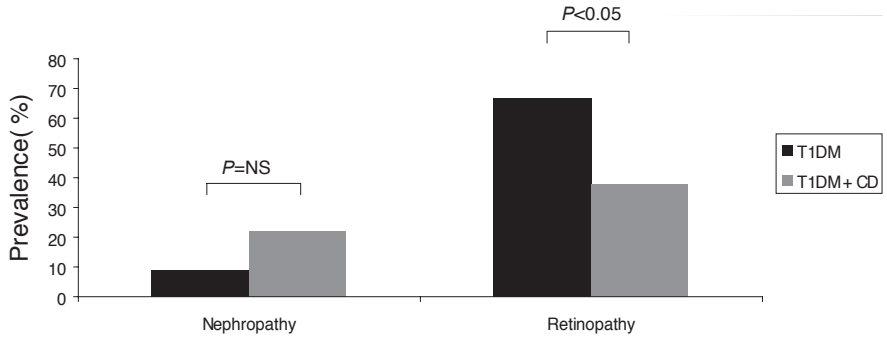


Table 2: Grade of retinopathy from T1DM patients with fundus photos. PDR= Proliferative diabetic retinopathy.

	Coeliac cases		Control subjects	
Grade	**n**	**%**	**n**	**%**
None	18	58.1	14	30.4
Mild	1	3.2	11	23.9
Moderate	4	12.9	3	6.5
Severe	0	0	2	4.3
PDR	7	22.6	15	32.6
Unable to grade	1	3.2	1	2.2
Total	31	100	46	100

Discussion:

In this national survey retrospective cross sectional study we report a lower prevalence of retinopathy in T1DM patients diagnosed at adult age with and treated for CD diagnosed at adult age, compared to matched controls. We found that the diagnosis of CD and treatment thereafter with a GFD was not of significant influence on glycaemic control. In addition, lower total cholesterol levels were observed in T1DM patients with concomitant CD.

Recently, the effects of long term treated CD in T1DM patients on diabetic complications received much attention in the literature. Picarelli et al reported about a potential protective role of diagnosed and treated CD in diabetic patients and found lower HbA1c levels together with less nephro- and retinopathy, which is partly in line with our findings[21]. On the contrary, it was reported that in T1DM patients with a mean duration of CD of 8.5 years, carotid intima media thickness was elevated compared to T1DM patients[16].

In our study no difference was found in the presence of microalbuminuria. This confirms and extent previous findings by Skovbjerg and colleagues in a large Danish T1DM cohort[23]. In 19 T1DM + CD patients they found no statistical difference in the prevalence of diabetic nephropathy compared to T1DM patients without CD. In contrast, in T1DM patients with undiagnosed CD a higher prevalence of retino- and nephropathy was observed[14]. These conflicting results could be due to the undiagnosed CD status of the patients in the latter study and therefore the lack of GFD. In addition, the management of CD in T1DM patients by a GFD have been found to confer a degree of reno-protection in these patients[15].
In our T1DM patients treated for CD a trend towards lower total and LDL cholesterol levels were found compared to controls. This is in line with previous studies in which CD patients with or without T1DM had also decreased levels of total cholesterol[21,22]. This suggests that GFD may result in improvement of lipid levels.

The conflicting outcomes in the different studies attempting to assess the influence of CD on diabetic complications, so far, may relate to factors such as small sample size, cross sectional design, the difference of newly diagnosed CD versus treated CD patients or due to the fact that a different matched control group was used.

The mechanism by which the presence of CD might affect HbA1c levels remains to be established. Hypothetically, a greater dietary vigilance in T1DM patients with CD might probably lead to healthier choices in eating. The raised hypothesis of lower advanced glycation end (AGE) products in GFD which might result in less microvascular complications asks further investigations[15]. Malabsorption of nutrients by small bowel mucosa before and after CD onset and the slow recovery of the mucosa after start of GFD might relate to lower HbA1c and cholesterol levels. However, data regarding the impact of dieting on metabolic control in patients with T1DM and CD cannot be considered unanimously positive[24]. Further, the disease activity of CD by itself might for unknown reasons contribute to the decreased prevalence of retinopathy. It might be plausible that an increased awareness of food intake and several consultations by a skilled dietitian might result in a better controlled carbohydrate intake.

Several limitations apply to our study. This was a retrospective, observational study and therefore associations may not reflect causality. Glycaemic control in our study is based on the most recent HbA1c levels. New insights have shown that glycaemic

variability might be a better marker for glycaemic control which contributes to the risk of diabetic complications, however, this is controversial and remains an active area of research[25,26]. In this retrospective study, continuous glucose monitoring (CGM) data was not available.
Moreover, diabetic complications by means of retinopathy and nephropathy are based on yearly and defined characteristics, and were prospectively measured. Although all patients were admitted to a GFD for at least one year, their compliance was not consistently confirmed by determination of serum antibodies against TTG or endomysium (EMA).

In conclusion, our results seem to indicate a protective role of concurrent (treated) CD in the development of retinopathy in T1DM patients and it emphasizes the awareness for CD in case of symptoms suggestive for CD in T1DM patients. This current observational study suggests that screening for CD in adults with T1DM might be mandatory to detect and treat CD in an early manner.

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Chapter 4

Advanced Glycation End products (AGEs) and the soluble Receptor for AGE (sRAGE) in Patients with Type 1 Diabetes and Coeliac Disease

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Abstract

Background: Advanced Glycation End (AGE) products play a role in the progression of diabetic complications. Gluten free diet (GFD) might affect AGE levels in patients who adhere to a GFD because of coeliac disease (CD). The aim of our study was to compare skin AGE levels and soluble receptor AGE levels (sRAGE) in patients with type 1 diabetes (T1DM) with (T1DM+CD) and without CD (T1DM-CD) and healthy controls.

Methods: We recruited 25 T1DM+CD and 25 T1DM-CD patients, matched for age, gender, diabetes duration, and glycaemic control alongside 25 healthy controls. We collected demographic, clinical and biochemical characteristics, including skin autofluorescence (AF), sRAGE and hs-CRP levels.

Results: The duration of T1DM in patients was 30±14 (+CD) and 29±14 years (-CD), whereas CD duration in T1DM+CD patients was 14±10 years. Skin AF levels in T1DM patients were higher compared to healthy controls (2.5±0.6 versus 1.9±0.4, $p<0.01$) and skin AF was independently associated with age ($r=0.72$, $p<0.01$). sRAGE levels were higher in T1DM-CD patients compared to healthy controls (1554 ± 449 versus 1309 ± 400, $p=0.049$) and independently associated with creatinine levels ($r=0.32$, $p<0.01$).

Conclusions: Our study demonstrates that skin AGE and sRAGE levels are elevated in T1DM patients compared with healthy controls. No difference in skin AF or sRAGE levels between T1DM patients with or without CD were observed. The present study suggests that differences in microvascular complications between T1DM and T1DM +CD patients are not due to differences in skin AF or sRAGE levels.

Introduction:

A pivotal role in the development and progression of diabetic complications in patients with type 1 diabetes (T1DM) is the formation of advanced glycation end (AGE) products[1]. AGEs are the result of the Maillard reaction and may be formed as a result of normal metabolism and aging. Under certain pathologic conditions, e.g. oxidative stress due to hyperglycaemia in patients with diabetes, AGE formation can be increased beyond normal levels. By the ambiguous presence of their receptors (RAGE), AGEs contribute to the development of atherosclerosis by increasing vascular permeability, inhibition of vascular dilatation by interfering with nitric oxide, oxidising low-density-lipoproteins, binding cells including macrophage, endothelial, and mesangial cells to induce the secretion of a variety of cytokines and enhancing oxidative stress[1,2]. In addition, it has been demonstrated that exogenously formed AGEs (e.g. heated food) are absorbed by the intestine into the bloodstream and represent a source of chemically active toxins[3].

Coeliac disease (CD) is one of the autoimmune disorders strongly associated with T1DM, with a prevalence of about 5% compared with approximately 1% in the general population[4]. CD is a permanent intolerance to ingested gluten that results in immunologically mediated inflammatory damage to the small intestinal mucosa and therefore leads to a malabsorption syndrome[5]. A gluten free diet (GFD) is the treatment of choice for CD and is low in high-temperature processed foods and in flour-based items, which are generally high in AGEs[6].

Recently, studies have addressed the question whether concomitant CD is of influence on microvascular complications in T1DM patients[6-12]. The role of (un) treated CD on the development of diabetic long term complications has been reported either to be protective[6,7,11] or to be aggravating[8,10,12].

The aim of the present study is to investigate AGE accumulation and serum sRAGE levels in T1DM patients with concomitant CD. We therefore identified patients with T1DM and with (T1DM+CD) who were treated by GFD and without concomitant CD (T1DM-CD). We measured AGE levels by skin autofluorescence (AF) and serum sRAGE levels and compared these results with healthy matched controls. Furthermore, we studied their association with low grade inflammation, measured as hs-CRP.

Methods:

Study population

In this observational multi-centre study performed between March 2012 and March 2013, we enrolled 75 age- and sex-matched individuals after informed consent of whom 25 with both T1DM and CD, 25 with T1DM only and 25 healthy controls. Healthy controls were self-reported healthy individuals working at the VU University Medical Centre. In addition, patients were matched by diabetes duration and $HbA1_c$ values. CD was diagnosed according to the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) criteria and T1DM according to the American Diabetes Association (ADA)[13,14]. Concomitant inclusion criteria for the T1DM + CD group was treatment with GFD for at least 5 years. Adherence to GFD was evaluated by a food questionnaire and by serological determination of tTG2. All T1DM + CD patients were recruited with help from 12 local and 2 academic hospitals in the Netherlands. The T1DM control group was selected from T1DM patients who visited the outpatient clinic of the Medical Centre Alkmaar.

The study was approved by the Ethical Committee of both the VU University Medical Centre and the Medical Centre Alkmaar.

Clinical characteristics

History taking consisted of questions regarding age, diabetes duration, duration of CD, duration of GFD, current or past tobacco use, insulin dose (U/kg/day), medication use (including cholesterol and blood pressure lowering therapies) and comorbidity. Physical examination consisted of measurement of weight, length and blood pressure. Body mass index (BMI) was defined as the individual's body weight divided by the square of his or her weight. Exclusion criteria were no self-reported adherence to GFD in the T1DM+CD group and clinical signs of infection.

Laboratory assessments

For measurement of biomarkers in serum, venous blood was collected into plain sterile tube. Serum was separated and stored in 5 ml cryotubes at -20 C until batch laboratory assessment. Serum creatinine levels were determined by the automated clinical chemistry analyser (Roche/ Hitachi 912/MODULAR analysers: ACN 652). Estimated glomerular filtration rate (eGFR) was calculated from the Modification of Diet in Renal Disease formula[15]. Glycaemic control was investigated by retrieving

the most recent $HbA1_c$ levels from the case files. Antibodies against tissue transglutaminase 2 (tTG2) were measured by an in-house developed ELISA using human recombinant tTG2 (Diarect, Freiburg, Germany) as described previously[16]. Serum levels of sRAGE (R&D Systems, Minneapolis, USA) were measured using commercially available enzyme linked immunosorbent (ELISA) techniques, according to manufacturer's instructions. Briefly, a monoclonal antibody generated against the N-terminal extracellular domain of human RAGE was used to capture sRAGE from serum. Captured sRAGE was detected with a polyclonal anti-human sRAGE antibody. After washing, plates were incubated with streptavidin-horseradish peroxidase, developed with substrate, and OD450 was determined using an ELISA plate reader. The intra- and interassay coefficient of variation (CV) values for the sRAGE measurement were 4.8-6.2% and 6.7-8.2%, respectively. Serum levels of hs-CRP were measured using an immunoturbidimetric assay (Modular analytics P, Roche diagnostics, Mannheim, Germany) with a lower detection limit of 0.3 mg/L.

Assessment of skin AF

Skin AF was assessed by the AGE Reader™ (DiagnOptics B.V., Groningen, the Netherlands). In brief, the AGE Reader™ illuminates a skin surface of 4 cm^2 at the lower arm, guarded against surrounding light, with an excitation light source between 300 and 420nm (peak excitation ~350nm)[17]. Three non-invasively measurements per patient were taken at room temperature on the volar side of the dominant forearm, at around 10 cm below the elbow fold, with patient in a sitting position. Care was taken to perform the measurement at a normal skin site, i.e. without scars, lichenification or other skin abnormalities. Skin AF is calculated as the ratio between the total emission intensity (420-600 nm) and the total excitation intensity (300-420 nm), multiplied by 100, and is expressed in Arbitrary Units (AU)[18]. All skin AF measurements were performed by two persons, the authors A.C. and E.G. Previous performed validation study showed an intra-individual Altman error percentage of 5.03%, with skin AF measurements taken over 1 single day, and an Altmann error percentage of 5.87% for seasonal variation[17].

Statistical analysis

Continuous data are expressed as means (SD) or median with interquartile range (IQR) as appropriate. Continuous variables were compared using analysis of variance. Chi-square statistics were used for categorical variables. Univariate regression and multivariate regression analyses were performed for determination of independent

relationships of variables with skin AF or sRAGE levels. Variables shown to be related to skin AF or sRAGE in univariate analysis ($p<0.10$) were tested for their independent effect by multivariate logistic regression analysis. We calculated a sample size of 25 patients in each of the three subgroups using $\alpha=0.05$ (type I error) and $\beta=0.20$ (type II error), assuming a difference in mean skin AGE level of 0.4 and a standard deviation of 0.5 for the skin AF levels in each subgroup[19]. A two tailed probability of $p < 0.05$ was considered as statistically significant. Statistical analysis was performed using IBM Statistical Package for the Social Sciences (version 20.0).

Results:

Clinical characteristics

Demographic, anthropometric and biochemical data are depicted in table 1. No significant differences were observed between the T1DM+CD and T1DM-CD patients regarding gender, age, duration of T1DM, insulin dosage per kg per day and $HbA1_c$ value. Low tTG2 values suggested adherence to a GFD, although tTG2 values in serum were slightly elevated in 3 patients (range 8.9-16.3 U/mL). The other participants were negative for tTG2. The presence of concomitant autoimmune diseases other than CD did not differ between T1DM+CD and T1DM-CD patients (both 36%) and consisted of Hashimoto thyroiditis (n=13), Sjögren's syndrome (n=2), vitiligo (n=2) and Graves' disease (n=1). No significant difference in creatinine levels were observed between T1DM+CD and T1DM-CD patients (81 ± 34 versus 68 ± 12, p= 0.08).

Advanced Glycation End products

As shown in figure 1, a significant difference in skin AGEs was observed between T1DM patients with or without CD and healthy controls (2.46 ± 0.66 units versus 1.96 ± 0.42 units, $p< 0.01$ and 2.49 ± 0.63 versus 1.96 ± 0.42, $p<0.01$, respectively). However, there were no differences in skin AF values between the T1DM+CD and T1DM-CD patients (2.46 ± 0.66 AU versus 2.49 ± 0.63 AU, p=0.86, respectively). sRAGE levels were higher in T1DM-CD patients compared to healthy controls (1554 ± 449 pg/ml versus 1309 ± 400, p=0.049 pg/ml; respectively, Figure 2). Mean sRAGE level was lower in T1DM+CD patients compared to T1DM-CD, although not significant (1395 ± 467 vs 1554 ± 449, p=0.24). No other differences were observed between the three groups and sRAGE levels.

Table 1: Group characteristics of patients with type 1 diabetes mellitus (T1DM) with or without coeliac disease (CD), and healthy controls (HC). Data are presented as mean with standard deviation except for hs-CRP (median with interquartile range). ^ = P < 0.05 between T1DM and HC. * = P < 0.05 between T1DM + CD and HC.

	T1DM + CD	T1DM	Difference T1DM + CD versus T1DM	HC	Difference between 3 groups
	(n=25)	(n=25)	P	(n=25)	P
Women (%)	64	60	0.77	80	0.27
Age (years)	53 ± 16	55 ± 15	0.55	49 ± 9	0.23
Caucasian (%)	100	100	1	100	1
Duration of T1DM (years)	30 ± 14	29 ± 14	0.88		0.88
Age at diagnosis of T1DM (years)	23 ± 19	27 ± 13	0.45		0.45
Duration of CD (years)	14 ± 10		NA		
Age of CD diagnosis (years)	38 ± 16		NA		
Concurrent autoimmune diseases (%)	36	36	1	4	**<0.05^**
Active smoker and/or smoking history (%	0	16	0.11	8	0.11
Body Mass Index (kg/m²)	24.7 ± 4.3	24.1 ± 3.4	0.56	24.3 ± 3.5	0.80
Blood pressure (mm/hg)					
Systolic	126 ± 16	127 ± 11	0.73	135 ± 22	0.14
Diastolic	75 ± 7	75 ± 8	0.89	83 ± 14	**<0.05***
Insulin dose (u/kg/day)	0.56 ± 0.22	0.65 ± 0.32		.	0.23
Blood pressure lowering drugs (%)			0.58		
ACE-inhibitors	12	20			0.44
Diuretics	20	8			0.22
Calcium-antagonist	0	8			0.15
Statin use (%)	30	44	0.37		0.34
Creatinine (µmol/L)	81 ± 34	68 ± 12	0.08	66 ± 10	0.420
Estimated GFR (MDRD)	89 ± 3	98 ± 19	0.23	94 ± 15	0.39
HbA1c (mmol/mol)	57.3 ± 9.6	62.7 ± 12.9	0.10		0.11
hs-CRP (mg/L)	1.2 (0.3-2.4)	1.1 (0.5-2.0)	0.37	2.1 (0.6-4.1)	0.51

NA= not applicable

Figure 1: Comparison of skin autofluorescence (AF) levels between patients with type 1 diabetes (T1DM) + coeliac disease (CD), T1DM patients and healthy controls are shown. Horizontal line represents the mean. AU: arbitrary units.

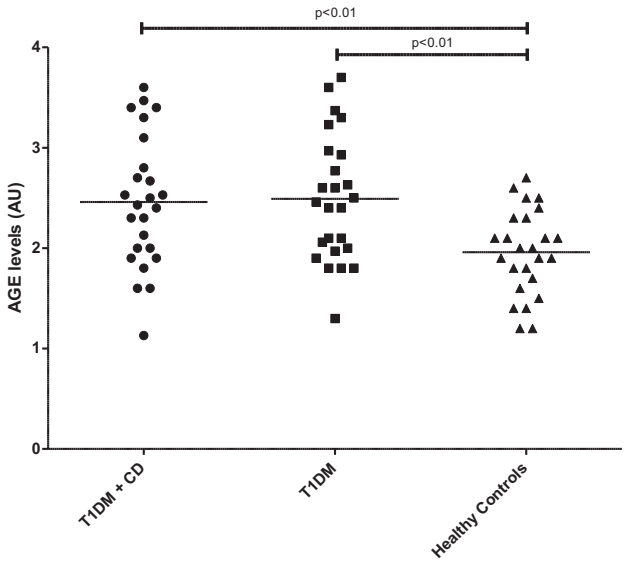
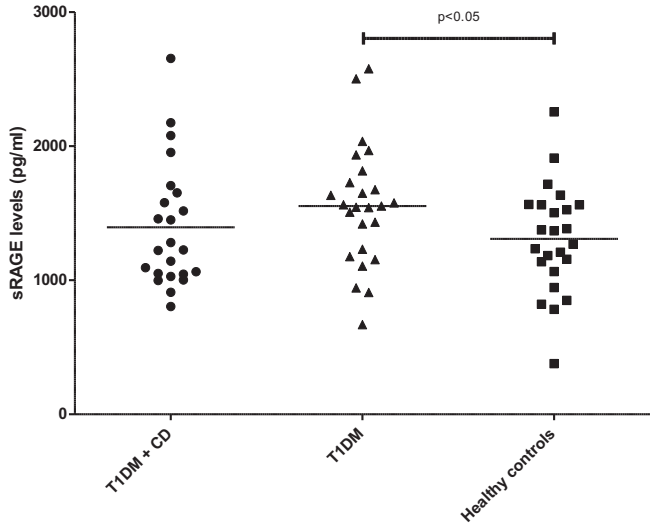


Figure 2: Comparison of soluble Receptor AGE levels (sRAGE) between patients with type 1 diabetes (T1DM) + coeliac disease (CD), T1DM patients and healthy controls are shown. Horizontal line represents the mean.



Uni- and multivariate analysis

The pooled univariate analysis comparing skin AF as the dependent variable and anthropometric and biochemical characteristics as independent variable showed a significant and direct correlation with age (r=0.716; p<0.01), estimated GFR_{mdrd} (r=0.407; p<0.01), creatinine (r=0.365; p<0.01), disease presence (T1DM-CD versus controls and T1DM+CD versus controls; r=0.395; p=0.002 and p=0.003), and $HbA1_c$ (r=0.268; p=0.062). However, in a multivariate model using the variables that significantly correlated in the univariate analysis (p<0.1) as covariates, only age resulted as an independent predictor of skin AF (Age: b=0.030; p<0.01; Table 2A).

The pooled univariate analysis for the 3 groups comparing serum sRAGE as the dependent variable and anthropometric and biochemical as independent variable showed a significant and direct correlation with creatinine (r=0.322; p<0.01) and estimated GFR_{mdrd} (r=0.232; p=0.055). In multivariate analysis, creatinine levels (b=10.403; p<0.01) and disease presence (T1DM-CD versus controls; b=243.2 p=0.046) resulted as independent predictors of sRAGE (Table 2B). Since creatinine levels significantly influence sRAGE levels we performed an analysis between T1DM + CD and T1DM patients correcting for creatinine levels. Regression analysis of sRAGE levels between patients with T1DM + CD versus T1DM revealed a significant effect on sRAGE levels (B=276.4; P=0.027). This indicates that sRAGE levels are lower in patients with both T1DM + CD after correction for creatinine levels.

Table 2:

A) Univariate and multivariate analysis (skin AF as dependent variable). Statistically significant values are showed with bold in the tables.

B) Univariate and multivariate analysis (soluble receptor AGE as dependent variable).

A)

Univariate and multivariate analysis (skin autofluorescence as dependent variable)

Variable	Univariate		Multivariate		
	r	Sig. (p)	*b*	Std.error	Sig (p)
Age	**0.716**	**<0.01**	**0.030**	**0.005**	**<0.01**
Estimated GFR (mdrd)	**0.407**	**<0.01**	0.001	0.005	0.912
Disease type (versus healthy controls)	**0.395**		-0.023	0.137	0.866
T1DM		**0.002**			
T1DM + CD		**0.003**			
Creatinine	**0.365**	**<0.01**	0.005	0.004	0.215
HbA1c	**0.268**	**0.062**	0.001	0.006	0.907
sRAGE	0.187	0.115			
Diastolic blood pressure	0.103	0.384			
Systolic blood pressure	0.093	0.434			
Smoking	0.089	0.445			
hsCRP	0.055	0.651			
BMI	0.021	0.860			

B)

Univariate and multivariate analysis (soluble receptor AGE as dependent variable)

Variable	Univariate		Multivariate		
	r	Sig. (p)	*b*	Std.error	Sig (p)
Creatinine	**0.322**	**<0.01**	**10.403**	**3.366**	**<0.01**
Disease type (versus healthy controls)	**0.232**				
T1DM		**0.055**	**243.186**	**119.471**	**0.046**
T1DM + CD		0.501			
Estimated GFR (mdrd)	**0.212**	**0.079**	2.371	3.204	0.462
Skin AF	0.187	0.115			
BMI	0.165	0.167			
HbA1c	0.184	0.215			
Smoking	0.132	0.271			
Diastolic blood pressure	0.117	0.334			
Age	0.075	0.529			
hsCRP	0.021	0.864			
Systolic blood pressure	0.003	0.979			

Discussion:

Several studies have shown that the concomitant presence of CD influences microvascular complications in T1DM patients[8-10,12-14]. In this cross sectional study we observed no differences in skin AF and sRAGE levels between patients with T1DM+CD compared to T1DM-CD patients. The strength of this study is the long duration of CD and GFD and the prospective design of this study. Our findings suggest that other mechanisms are responsible for the differences in microvascular complications between these groups. A GFD may lead to lower cholesterol levels and lower BMI, both traditional cardiovascular risk factors[11]. In addition, a recent study of Pham et al found that non-adherence to a gluten free diet was associated with an elevated albumin-excretion rate[20].

AGEs cause damage by intracellular glycation, cross-link formation, and interaction with specific cellular receptors, that is RAGE[21,22] . This receptor is a multiligand member of the Ig superfamily of cell surface molecules that engages AGEs and leads to cellular signaling, including nuclear factor kB (NF-kB) activation, increased cytokine and adhesion molecule expression, the induction of oxidative stress, and an increase in cytosolic reactive oxygen species[21]. The soluble RAGE isoform (sRAGE) expresses the cellular concentration of RAGE and reflects the total pool of soluble RAGE in plasma, which includes several variants. However, the precise role of sRAGE in the pathophysiology of T1DM and vascular complications remains unclear. Despite that, sRAGE levels might have clinical implications as two large studies of individuals with T1DM observed a positive association between sRAGE

levels and cardiovascular mortality[23,24] . Our study does partly confirm a previous study performed in children[25]. They also found higher sRAGE levels in T1DM patients versus healthy controls (1565±67 versus 1254±56 pg/mL). However, they observed lower sRAGE levels in T1DM +CD patients compared to the T1DM-CD group (1305±24 and 1565±67 pg/mL, respectively). Since sRAGE is independently and positively associated with renal dysfunction[23], we corrected for creatinine levels and found lower sRAGE levels in T1DM+CD patients. This suggests that GFD may have, at least some, cardiovascular protective properties in T1DM patients.

A recent large nationwide population study in Sweden found that duration of CD < 10 years besides T1DM revealed a protective effect for diabetic retinopathy while a longer duration of CD had an increased risk for diabetic retinopathy[10]. One of the limitations of the Swedish study was that clinical factors, including body mass index, glycaemic control, insulin dosage and adherence to GFD were not available. These findings however, were, confirmed in our present, but smaller study with inclusion of the above mentioned clinical factors. We observed a protective effect of concomitant CD with a mean duration of 3 years in patients with T1DM (IQR 1-8) for diabetic retinopathy[7]. Future measurements in patients with both T1DM and CD (duration < 10 years) should result, when our hypothesis is correct, in lower AGE levels when compared to patients with T1DM only.

No differences in skin AF levels were found between T1DM patients with or without CD, while we could confirm the higher skin AF levels in patients with T1DM compared to healthy controls[17]. Several studies have found that skin AF in T1DM patients is associated with microvascular complications [19,26,27]. It was shown that skin AF is increased in T1DM patients in relation to the severity of diabetic neuropathy and foot ulceration[27] and in patients with nephro-, neuro- and retinopathy[26]. As our T1DM+CD patients were on a long duration of CD (mean duration of 14 years), this suggests that a GFD might not affect skin measured AGE's.

It has been demonstrated previously that restriction of dietary AGEs in patients with T1DM reduces markers of oxidative stress and inflammation, including Tumor Necrosis Factor alpha (TNF-a), hs-CRP and vascular cell adhesion molecule 1 (VCAM-1)[28]. We did not observe a difference in hs-CRP levels between T1DM patients with or without a GFD. This suggests that a GFD does not influence hs-CRP levels in patients with T1DM, which is in line with previous findings[12].

Some limitations apply to our study. The main limitation is that the study size is small and therefore subtle changes may have been missed. It would be of interest to perform longitudinal studies and collect follow-up data on skin AF + sRAGE levels and on clinical events, including the development of microvascular complications. A potential factor influencing our skin AF results could have been the fact that our patients were not measured after an overnight fast[29]. Furthermore, skin AF is correlated with AGEs derived from skin biopsies: CLF, pentosidine, CML, and CEL (r=0.47–0.62, $p \leq 0.002$)[30]. However, skin AF is a group reactivity and cannot provide quantitative information on the concentrations of individual compounds.

In conclusion, the results of the present study shows that skin AGE and sRAGE levels are elevated in T1DM patients compared with healthy controls. However, we found no differences in skin AF and sRAGE levels between T1DM+CD and T1DM-CD patients.

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Chapter 5

Compromised quality of life in patients with both type 1 diabetes mellitus and coeliac disease

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Abstract

Background: Type 1 Diabetes Mellitus (T1DM) and Coeliac Disease (CD) are two chronic illnesses associated with each other. Both diseases and their treatments can seriously impair quality of life (QOL). Objective of the present study is to investigate health related QOL in adult patients diagnosed with both T1DM and CD and compare this with healthy controls and controls who have T1DM only.

Methods: A generic measure of health related QOL (RAND-36) and a measure of diabetes specific QOL (DQOL) questionnaire were sent to patients diagnosed with both T1DM and CD. The control group consisted of patients with T1DM without CD matched for age, gender and socioeconomic status. Generic QOL scores were compared with data from healthy Dutch controls.

Results: Fifty-seven patients with T1DM and CD were included and no associations between clinical characteristics and QOL were observed. Women reported a lower QOL in social functioning, vitality and mental health than men (all $P<0.05$). A lower DQOL was observed regarding diabetes related worries and social worries in patients with T1DM and CD compared to patients with T1DM. Compared with healthy controls, QOL in patients with T1DM and CD was significantly lower, particularly social functioning (Cohen's $d = 0.76$) and general health perception (Cohen's $d = 0.86$).

Conclusions: The additional diagnosis of CD and treatment by gluten free diet in adult patients with T1DM has a considerable, negative impact on QOL and DQOL. Women are particularly affected and social functioning and general health perception is compromised.

Introduction:

Patients with Type 1 Diabetes Mellitus (T1DM) are at increased risk of developing micro- and macrovascular complications and cardiovascular disease[1]. Subsequently, the health related quality of life (QOL) in people with T1DM is compromised which is associated with having diabetic complications and poor glycaemic control[2,3]. Patients with T1DM have an increased risk of developing other autoimmune disorders, particularly coeliac disease (CD) and autoimmune thyroiditis[4]. CD is a permanent intolerance to ingested gluten and leads to a malabsorption syndrome[5]. The only treatment for CD is a strict adherence to a gluten free diet (GFD) which is of influence on QOL[6]. The GFD by excluding foods containing wheat, rye, barley and oats is a lifelong treatment and is considered to be of influence on QOL[6]. Both T1DM and CD are chronic illnesses which influence QOL since the treatments are burdensome and the complications can be debilitating and life threatening. Data are lacking about the impact of these two diseases on the QOL in those patients.

Therefore, the aim of the present study is to investigate generic and diabetes specific QOL in patients with both T1DM and CD. Further, we investigated whether (clinical) characteristics of patients with both T1DM and CD were associated with generic or diabetes related QOL.

Patients and methods:

Patients and controls

By advertisement in the journals of the Dutch Coeliac Disease Society, the Dutch Diabetes Society and by help of physicians, patients with T1DM and concomitant CD were asked to participate in this observational study in adults. Inclusion criteria included: age (>18 years), reported biopsy proven coeliac disease, and self-reported compliance to a GFD. T1DM diagnosis was according to the American Diabetes Association position statement[7]. The participants of the control group were patients with T1DM without CD and recruited from two local hospitals. The control group was matched for age, gender and socioeconomic status. The scores of the respondents were compared with published reference values of a Dutch population based sample of 1063 adults[8]. The medical ethical committee of the VU University Medical Centre approved both the study and the consent form (NL 40438.029.12).

Clinical data

The survey included questions concerning socio-economic status, age of onset of T1DM, age of diagnosis of CD, complaints at diagnosis of CD, duration of CD complaints before diagnosis, date of start of GFD, and (autoimmune) comorbidity (e.g. autoimmune thyroiditis, Addison's disease).

Quality of life (QOL)

Generic QOL

The RAND-36 is composed of 36 questions and organized into the following nine multi-item scales: physical functioning, social functioning, role limitations as a result of physical health problems, role limitations as a result of emotional problems, general mental health, vitality, bodily pain, general health perceptions and health change[8,9]. Higher scores indicated higher levels of functioning or well-being (scores vary between 0 to 100)[10]. In a Dutch validation study, Cronbach's α of the nine scales of the RAND-36 had high degrees of internal consistency (Crohnbach's α = 0.71-0.92) and good test-retest reliability (r = 0.58-0.82)[8].

DQOL-Questionnaire:

The DQOL questionnaire contains 46 items which the subjects rank on a 5-point Likert scale[11]. A score of 1 represents no impact or worries and always satisfied and a score of 5 represents always affected, worried or never satisfied. Cronbach's α, was tested before and its four scales had high degrees of internal consistency (Crohnbach's α = 0.66-0.92) and excellent test-retest reliability (r = 0.78-0.92)[11].

Data analysis

Mean differences of continuous variables between the study cohort and the reference control group were assessed using the Student's t-test. For dichotomous variables, the Fisher-exact test or Chi-square test was used. Cohen's d was calculated for each of the implicit and explicit measures for each group (small, medium, large effect size)[12]. A two tailed probability of $P < 0.05$ was considered as statistically significant. Statistical analysis was performed using Statistical Package for the Social Sciences (IBM, version 20).

Results:

Sociodemographic and clinical characteristics

A total of 74 patients were eligible to participate and surveys from 57 patients with T1DM and CD were returned (77% response rate). In this cross sectional study, the mean age of the patients with T1DM and CD was 49 ± 16 years (Table 1). No significant differences were seen between the two groups regarding socio economic variables. A third autoimmune disease was present in 36% of patients with both T1DM and CD and in 21% of the controls.

General HR-QOL

Patients with both T1DM and CD

Women had a significantly lower score on the subscales social functioning (65 ± 22 versus 83 ± 27, P=0.009, Cohen's *d* = 0.69), vitality (55 ± 17 versus 68 ± 20, P=0.021, Cohen's *d* = 0.68) and mental health (68 ± 19 versus 80 ± 15, P=0.01, Cohen's *d* = 0.67), compared to men. Reported delay in CD diagnosis, type of CD complaints or the presence of another autoimmune disease were not significantly associated with the different aspects of HR-QOL (data not shown).

Patients with both T1DM and CD versus patients with T1DM

The subscores of the RAND-36 did not significantly differ between the two groups. The social functioning score in the patients with T1DM and CD was 72 ± 26 versus 79 ± 25 in the patients with T1DM (P=0.16).

Patients with both T1DM and CD versus healthy controls

Five subscores were significantly lower in the patients with both T1DM and CD (Fig. 1). The largest effect size was observed in the subscores general health perception (53 ± 25 versus 73 ± 3, P< 0.0001, Cohen's *d* = 0.87) and social functioning (71 ± 26 versus 87 ± 21, P< 0.0001, Cohen's *d* = 0.76). Further, the subscores vitality 59.6 ± 19.3 versus 67 ± 20, P=0.0063, Cohen's *d* = 0.40), role limitations as a result of physical health problems (65 ± 45 versus 79 ± 36, P=0.0034, Cohen's *d* = 0.39) and role limitations as a result of emotional problems (74 ± 40 versus 84 ± 32, P=0.024, Cohen's *d* = 0.31) were lower in patients with both T1DM and CD (Fig. 1).

Table 1: Comparison of demographic and clinical characteristics of adults with type 1 diabetes (T1DM) and coeliac disease (CD) and matched control patients with T1DM. Data are presented as mean with standard deviation (±). Lower diabetes specific quality of life score indicates better quality of life.

	T1DM + CD (n=57)	T1DM (n=57)	P-Value
Women (%)	60%	46%	0.13
Age (years)	48 ± 16	52 ± 14	0.17
Age of onset of T1DM (years)	22 ± 16	26 ± 14	0.17
Duration of T1DM (years)	25 ± 16	26 ± 15	0.72
Age of onset of CD (years)	39 ± 16		
Duration of CD (years)	9 ± 10		
HbA1c (mmol/mol)	61 ± 13	60 ± 13	0.57
HbA1c (%)	7.7 ± 1.2	7.6 ± 1.2	0.57
Body Mass Index	25 ± 4	25 ± 4	0.29
Concurrent autoimmune diseases (%)	36%	21%	0.07
Complaints at CD diagnosis (%)			
Gastrointestinal complaints	55%		
Anaemia	20%		
Hypoglycaemia	5%		
No complaints	20%		
Duration of CD complaints* (%)			
< 6 months	15%		
6-12 months	20%		
1-5 years	20%		
> 5years	45%		
Education level			0.11
Primary school	25%	30%	
Basic high school	30%	35%	
Advanced high school	28%	32%	
University	18%	4%	
Marital status			0.94
Single	18%	16%	
Divorced	7%	7%	
Married	75%	77%	
Employment status			0.22
Unemployed	13%	13%	
Part time	41%	25%	
Full time	25%	41%	
Retired	21%	21%	
Living area			0.09
Country side	32%	47%	
City	68%	53%	
Smoking history			0.19
Yes	11%	19%	
Diabetes specific quality of life			
Impact	2.1 ± 0.47	2.0 ± 0.42	0.29
Satisfaction	2.5 ± 0.67	2.4 ± 0.74	0.27
Worry: diabetes related	2.0 ± 0.77	1.7 ± 0.56	0.003
Worry: social/ vocational	1.8 ± 0.70	1.4 ± 0.49	0.001

* Patients without any reported complaints at CD diagnosis were excluded from this specific analysis

DQOL

Patients with both T1DM and CD

Women scored higher on the worries diabetes subscale than men (2.2 ± 0.8 versus 1.8 ± 0.6, P< 0.05, Cohen's *d* = 0.57, respectively). No other differences in the three subscales between men and women were observed.

Patients with both T1DM and CD versus patients with T1DM

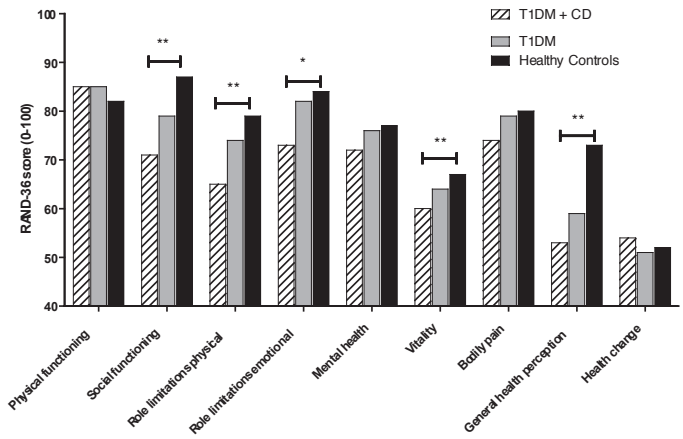
The subscales worries social and worry diabetes related were significantly higher in the group of patients with both T1DM and CD (Table 1). The effect size for worry social/vocational was Cohen's $d = 0.63$ and for worry diabetes related Cohen's $d = 0.43$.

Discussion:

In this cross sectional study we found that adult patients with T1DM also diagnosed with and treated for CD, reported lower QOL in several domains compared to patients with T1DM or healthy controls.

QOL in paediatric patients with T1DM and CD have been compared with paediatric patients with T1DM and no differences in QOL were observed in a study by Sud et al[13]. However, lower social function was found in the parents of children with T1DM and CD. Difficulties with social functioning have been reported before in patients with CD[14]. These findings consistently suggest that concomitant CD impairs social aspects of life in people with T1DM. We did not observe these differences in social functioning by using the RAND-36 but observed this with the DQOL. This might be related to the more disease specific questions of the DQOL[15].

Figure 1: Comparison of RAND-36 of adults with type 1 diabetes mellitus (T1DM) and coeliac disease (CD) (n=57) with patients with T1DM (n=57) and with Dutch individuals from the general population (n=1063)[11]. Data are presented as mean. Higher scores indicate better QOL. * P < 0.05, ** P < 0.01.



An impaired QOL is associated with the prevalence of depression in diabetes patients and it is therefore of importance to recognize both[16]. Garud et al found a markedly elevated risk of depression in patients with both T1DM and CD[17]. In their study, the prevalence of depression was 37% in patients with T1DM and CD and 17% in patients with CD[17]. This data suggests, together with our findings, that these patients with T1DM and CD, particularly women, are at higher risk for depression.

Several limitations apply to our study. Selection bias in this study might exist since patients were recruited by their treating physicians and by help of the CD and T1DM society and this may not be representative of the population with T1DM and CD. Further, the control group of patients with T1DM were recruited from two other hospitals. Moreover, we did not address adherence to a GFD by serology, dietary interviews or food records and differences in QOL might have been influenced by adherence to a GFD[18]. In addition, determination of the exact CD related complaints at diagnosis is difficult (ie extraintestinal presentation)[4].

In summary, results of the present study show that patients with both T1DM and CD report a compromised QOL in comparison with patients with T1DM and healthy controls. Particularly women, and social functioning and general health perception is affected.

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Chapter 6

Contrasting the Genetic Background of Type 1 Diabetes and Coeliac Disease Autoimmunity

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Abstract

Background: Type 1 diabetes (T1D) and coeliac disease (CeD) cluster in families and can occur in the same individual. Genetic loci have been associated with susceptibility to both diseases. Our aim was to explore the genetic differences between individuals developing both these diseases (double autoimmunity) versus those with only one. We hypothesized that double autoimmunity individuals carry more of the genetic risk markers that are shared between the two diseases independently.

Methods: SNPs were genotyped in loci associated with T1D (n = 42) and CeD (n = 28) in 543 subjects who developed double autoimmunity, 2,472 subjects with T1D only, and 2,223 CeD-only subjects. For identification of loci that were specifically associated with individuals developing double autoimmunity, two association analyses were conducted: double autoimmunity versus T1D and double autoimmunity versus CeD. HLA risk haplotypes were compared between the two groups.

Results: The CTLA4 and IL2RA loci were more strongly associated with double autoimmunity than with either T1D or CeD alone. HLA analyses indicated that the T1D high-risk genotype, DQ2.5/DQ8, provided the highest risk for developing double autoimmunity (odds ratio 5.22, P = 2.25 × 10(-29)).

Conclusions: We identified a strong HLA risk genotype (DQ2.5/DQ8) predisposing to double autoimmunity, suggesting a dominant role for HLA. Non-HLA loci, CTLA4 and IL2RA, may also confer risk to double autoimmunity. Thus, CeD patients who carry the DQ2.5/DQ8 genotype may benefit from periodic screening of autoantibodies related to T1D.

Introduction:

Type 1 diabetes (T1D) and coeliac disease (CeD) are immunologic disorders, affecting between 0.5% and 1% of the general population[1,2]. They are both multifactorial diseases arising from a combination of multiple genetic and environmental factors. In addition, these two diseases co-occur in families, and even in single patients, more often than expected by chance[3]. Approximately 4–9% of patients with T1D also have CeD[4], while patients with CeD are at increased risk of developing T1D[5]. Since the genetic contribution within each disease is high, there may be an overlap in their etiology due to shared genetic risk factors[6] or due to synergistic effects of the genes involved in each disease separately[7].

Both T1D and CeD are seen mainly in populations of European ancestry, although they occur at a lower prevalence in African, Asian, and Latin American populations[2,8,9]. The underlying autoimmune processes share some features, but the autoreactive T cells and autoantibodies are directed against different autoantigens: insulin, GADA65, and IA-2 in T1D and tissue transglutaminase and endomysial antibody in CeD[10]. In most patients, preislet and celiac autoimmunity develop in early childhood, although both diseases can also develop later in life[11,12].

The class II genes explain a major component of familial clustering in both T1D and CeD, in particular the HLA-DRB1, HLA-DQA1, and HLA-DQB1 genes[13]. For T1D, alleles of HLA class II genes can confer both disease susceptibility and disease protection. Individuals carrying both the DR3-DQ2 (DRB1*03-DQB1*0201) and DR4-DQ8 haplotype (DRB1*04- DQB1*0302) are at the highest risk for developing T1D[14]. Its presence marks a 55% risk of developing overt diabetes by age 12 years[15]; however, only 20–50% of patients with T1D carry this genotype. For CeD, the most prominent association is with HLA-DQ2.5 (DQA1*0501-DQB1*0201)[16]. Individuals homozygous for the DQB1*02 allele (i.e., carriers of DQ2.5/DQ2.5 and DQ2.5/DQ2.2) are at high risk of developing CeD[17]. Genome-wide association studies (GWAS) have revolutionized the identification of additional predisposing risk factors to these diseases outside the HLA region. To date, more than 40 non-HLA loci for T1D and 26 non-HLA loci for CeD have been identified by GWAS (summarized at www.t1dbase .org[18-20]). It is noteworthy that many of the non-HLA loci are shared between various autoimmune diseases [7,21]. GWAS and cross-disease studies have identified the same regions, or even the same single nucleotide polymorphisms

(SNPs), as associated with both T1D and CeD, including the HLA, TAGAP, IL18RAP, SH2B3, CTLA4, CCR5, IL2/21, BACH2, UBASH3A, and PTPN2 loci[7,22].

Individuals affected by more than one autoimmune disorder may have an immune response more disturbed than those with only one disease. Specific genetic factors already identified as contributors to risk of T1D and CeD individually could be critical for double autoimmunity. Thus, our aim was to examine the genetic differences between individuals developing both T1D and CeD with respect to the genetic risk associated with having only one of these diseases.

Research design and Methods:

Patients and control participants

Patients and Control Participants Informed consent was obtained for all samples used, and the project was approved by the ethics committees of each of the institutions involved. T1D-only samples were collected from the Type 1 Diabetes Genetics Consortium (T1DGC), and CeD-only samples were collected from previous studies[19,23,24]. Samples from individuals with both T1D and CeD (double autoimmunity) were collected from T1DGC, the Barbara Davis Center, and the VU University Medical Centre (Amsterdam, the Netherlands) (Table 1). The identification of T1D only was based on self-reports, evaluation of medical records, and, when indicated, C-peptide determination using a standard protocol of the T1DGC. The identification of double autoimmunity individuals among patients first diagnosed with T1D was based initially upon self-reporting and confirmed by having high and persistent levels of IgA transglutaminase (IgA tissue transglutaminase) autoantibodies or confirmed by biopsy[25]. T1D was identified in patients first diagnosed with CeD according to the guidelines of an American Diabetes Association position statement[26]. The patients with CeD only were identified with autoantibody testing, confirmed by an intestinal biopsy[27]. Control subjects of Caucasian ancestry were also included[23]. In total, 543 individuals with double autoimmunity were identified, 3,098 patients with T1D only, 12,480 CeD-only patients, and 11,023 control subjects. All samples were genotyped using the ImmunoChip[23]. The hybridization and processing of the CeD samples and part of the double autoimmunity samples (those not from T1DGC) were performed in the Department of Genetics, University Medical Centre Groningen (UMCG), while the

genotyping of the T1D samples and the double autoimmunity samples from T1DGC was performed at the Genome Sciences Laboratory in the Center for Public Health Genomics at the University of Virginia. A total of 28 non-HLA SNPs associated with CeD and 42 SNPs with T1D were selected, all at genome-wide significance[19,20,23,28-33] ($P < 5 \times 10^{-8}$). After quality control, 66 SNPs remained for our analysis: 21 non-HLA SNPs associated with CeD-only, 33 SNPs associated with T1D-only, and 12 SNPs from eight loci shared between the two diseases. For prediction of whether an individual carries HLA-DQ2 (DQ2.5 or DQ2.2) and/or DQ8 alleles, five of the six tagging SNPs described by Monsuur et al. [34] were used. We failed to predict the HLA-DQ7 haplotype, as the sixth SNP (rs4639334) failed quality-control metrics.

Table 1: The samples and datasets used in our analyses

Origin (by Center)	Double autoimmunity Cases	Type 1 diabetes-only Controls	Celiac disease-only Controls	Total
Barbara Davis Center	313	-	-	313
T1DGC	147	2472	-	2619
Free University of Amsterdam	51	-	-	51
UMCG (The Netherlands)	32	-	2223	2255
Origin (by Country)				
United States	460	2472	-	2932
The Netherlands	83	-	1134	1217
United Kingdom	-	-	1089	1089

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Study Groups and Quality Control

Two data sets were assembled and two independent analyses performed to identify SNPs contributing to double autoimmunity. Individuals in the first analysis consisted of "case" subjects with double autoimmunity and "control" subjects with T1D only (T1D+CeD/T1D). Individuals in the second analysis consisted of "case" subjects with double autoimmunity and "control" subjects with CeD only (T1D+CeD/CeD). The quality-control assessment protocols were conducted for each study group independently. Individuals were excluded with call rate < 99.5% or sex inconsistency or if there was a first- or second-degree relationship with the index case. SNPs were excluded with a genotyping rate < 99%, minor allele frequency <0.05%, and failure of Hardy-Weinberg equilibrium assumptions ($P < 5 \times 10^{-6}$). The latter analysis was performed using KING, version 1.4, software[35]. Owing to the different ethnic backgrounds present in the sample (samples from North America, Europe, U.K., and Asia Pacific in the T1DGC data set and from Europe and India in the CeD

data set), a principal components analysis was applied to each of the data sets with the aim of identifying and excluding possible ethnicity outliers and to reduce the possibility of population stratification. This analysis was performed sequentially using EIGENSTRAT, version 4.2, software[36] and removing outliers at each step. After quality control, the data set included 2,955 individuals for the T1D+CeD/T1D analysis (1,451 males and 1,504 females) and 2,655 individuals for the T1D+CeD/CeD analysis (865 males and 1,790 females) all the samples with Caucasian origin.

Statistical Analysis

The association analysis was conducted separately for HLA and non-HLA risk loci. For the HLA locus, the analysis was performed on the predicted haplotypes and genotypes of DQ2.5 (DQA1*0501, DQB1*0201, and DRB1*03), DQ2.2 (DQA1*0201, DQB1*0202, and DRB1*07), and DQ8 (DQA1*03, DQB1*0302, and DRB1*04) and including the first five principal components as covariates. These haplotypes are well-known risk factors for both T1D and CeD. The absence of any of these haplotypes was classified as "other." The HLA analyses were divided into an analysis of the number of haplotypes per individual (whether an individual was carrying 0, 1, or 2 copies of the tested haplotype) and of genotypes (whether an individual was carrying combinations of risk haplotypes). Association analyses were performed for each study group using a genetic-based matching score. Pairwise comparisons of identity by descent were calculated for all samples, and then individuals were matched and clustered in homogeneous groups of case and control subjects to reduce false-positive associations owing to population stratification. With the results from the calculated clusters, a Cochran-Mantel-Haenszel analysis was performed, correcting the association for the genomic control inflation factor (λ).
Nominal statistical significance of $P < 0.05$ was used as the threshold for association, as the analyzed SNPs had been associated in previous GWAS and replicated at genome-wide significance. The analyses were performed using PLINK, version 1.07, and the statistical suite R, version 3.1.0[37,38].

Results

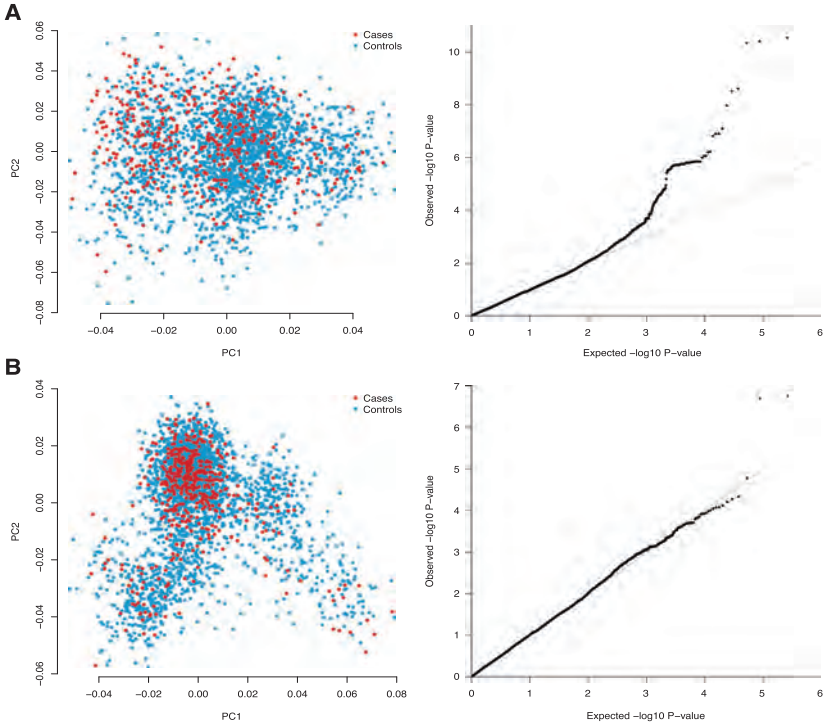
After completion of quality-control procedures and removal of outliers, a total of 2,955 samples (483 case and 2,472 control subjects) were included for the first T1D+CeD/T1D analysis; a total of 2,655 samples were included for T1D+CeD/CeD analysis (432 case and 2,223 control subjects). There was no evidence of

significant inflation in the results of association ($\lambda_{T1D+CeD/T1D}$ = 0.99; $\lambda_{T1D+CeD/CeD}$ = 1.04) for either set of analyses (Fig. 1).

Comparing Known Risk Alleles Across Diseases

We first aimed to investigate the status of established CeD and T1D loci across the published GWAS data sets[19,20,28-33] considering only those loci with at least one reported risk allele–associated genome-wide significance ($P < 5 \times 10^{-8}$) and confirmation in independent samples. Across 28 non-HLA SNPs from CeD and 42 non-HLA SNPs from T1D, representing 60 distinct risk loci, eight loci (represented by 12 SNPs) are shared between both diseases. Four of the reported SNPs in CeD and/or T1D (rs13010713-*ITGA4*, rs11755527-*BACH2*, rs1265564-*CUX2*, and rs917997-*IL18RAP*) were removed based on quality-control metrics, with one SNP proxy inserted (rs917997 was replaced by rs7559479 for *IL18RAP*). In total, 66 SNPs were included in the association analysis.

Figure 1. Principal components and Q-Q plot of each group of analyses. The principal components analysis was used to cluster the most homogenous samples for association analysis. The shape of the clusters differs because of the different origins of the merged samples, however, it is still possible to observe a good match between cases and controls. A. Double autoimmunity versus celiac disease-only patients. B. Double autoimmunity versus patients with T1D only. PC1. principal component 1; PC2; principal component 2.



Genetic Association in Double Autoimmunity Patients

Results of the association analysis for each of the 66 SNPs that passed our quality control in the two diseases are shown in Fig. 2 (odds ratio [OR] and 95% CI). Of the 21 CeD-only SNPs, 6 (28.6%) were associated ($P < 0.05$) with risk of double autoimmunity (Table 2). Similarly, of 33 T1D-only SNPs, 8 (24.2%) from six loci were associated ($P < 0.05$) with double autoimmunity (Table 3).

Of the 12 SNPs in eight loci that were shared across T1D and CeD, 10 SNPs (representing seven loci) exhibited the same trend of effect compared with the effect on individual disease risk in previous T1D or CeD GWAS. The *IL12A* locus SNP rs17810546 had an opposite effect in the double autoimmunity group (ORT1D+CeD/CeD 0.72; minor allele frequency 0.16, $P = 0.022$) than in the CeD GWAS (OR 1.36). There was only one locus shared between T1D and CeD (*CTLA4*[rs3087243]) that was associated in both T1D+CeD/T1D ($P = 0.001$) and T1D+CeD/CeD ($P = 0.0006$). The association of double autoimmunity with *IL2RA* differed in the SNP for the two groups, with rs61839660 in T1D+CeD/CeD ($P = 0.001$) but rs12251307 in T1D+CeD/T1D ($P = 0.0175$). These two SNPs are in linkage disequilibrium ($r2 = 0.543$, $D' = 0.84$); however, rs61839660 is located intronic in *IL2RA*, while rs12251307 is 5' of the same gene.

Association of HLA Loci

None of the HLA haplotypes (*HLA-DQ2.5*, *HLA-DQ2.2*, or *HLA-DQ8*) were statistically significant for association of double autoimmunity with respect to CeD only (T1D+CeD/CeD). The *HLA-DQ8* haplotype had the highest risk for double autoimmunity, though not significant, when the double autoimmunity individuals were compared with those with CeD only (OR 5.09, $P = 0.16$). In contrast, the *HLA-DQ2.5* haplotype was significantly associated ($P = 0.0003$) with double autoimmunity relative to T1D only (OR 1.44). There was absence of association of double autoimmunity with "other" HLA risk haplotypes (Table 4).

T1D+CeD/CeD analysis identified a significant association with the heterozygote genotype *DQ2.5/DQ8* (OR 1.47, $P = 3.31 \times 10^{-10}$) (Table 4). In the double autoimmunity group, we identified the haplotype *DQ2.5/DQ2.5* (OR 1.2, $P = 0.005$) as significantly associated with risk compared with T1D only (Table 4).

Figure 2: ORs and CIs for all the variants evaluated. ORs and 95% CIs for all the SNPs associated with CeD, T1D, or both that passed our quality controls. A: Double autoimmunity vs. T1D. B: Double autoimmunity vs. CeD. Highlighted markers correspond with those with a significant P value < 0.05. It was not possible to detect an enrichment of CeD or T1D variants associated with double autoimmunity based on the analysis of both data sets.

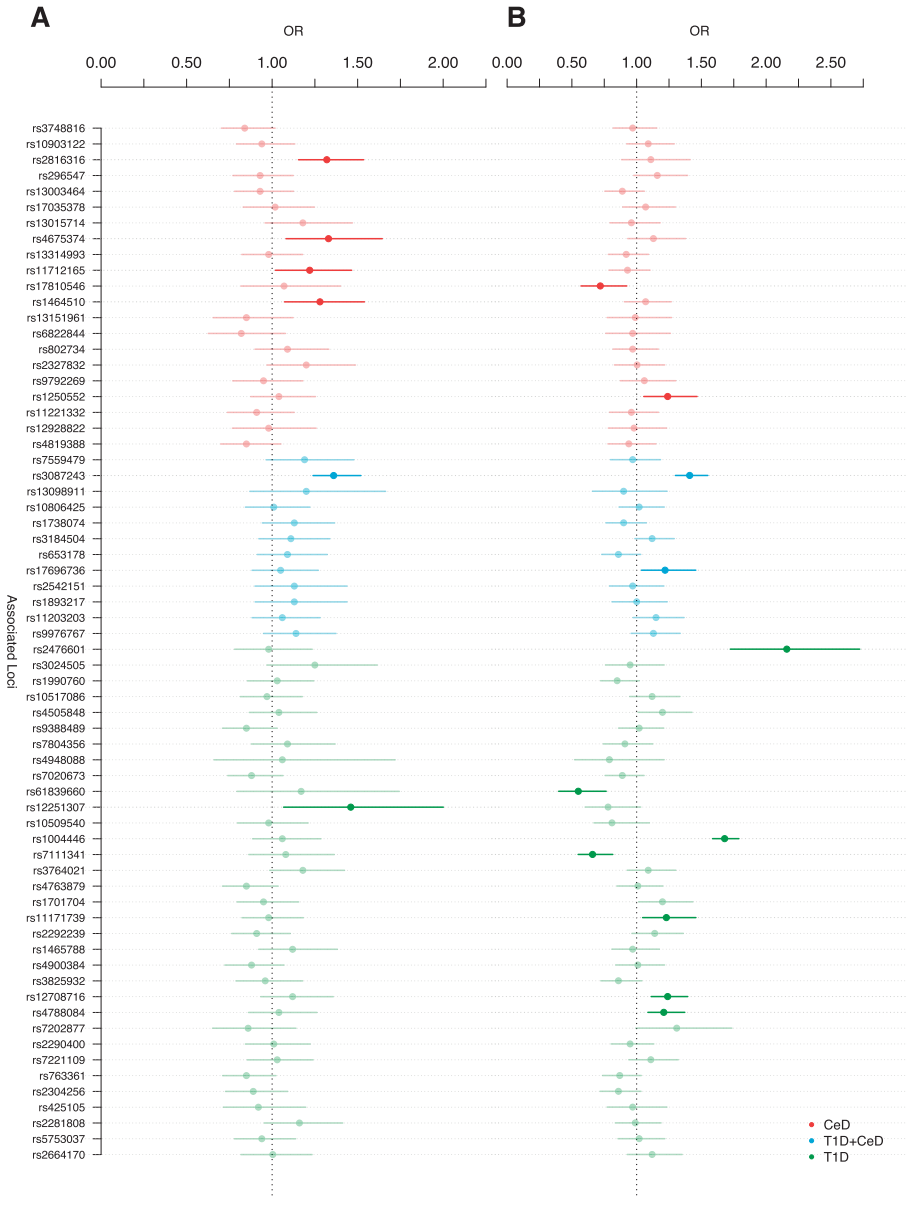


Table 2: Association of previously described variants for CeD disease and T1D in the dataset of double autoimmunity versus CeD-only patients. A1, allele associated; Allele freq., allele frequency for which OR is reported; Chr, chromosome; Gene reported, the most plausible gene reported by the literature; NR, not reported; Position in base pair; Ref, reference

Chr	SNP	Position	A1	Allele freq.	*P*	Reported Disease	Risk Allele	OR reported	*p* value reported	Gene reported	Ref
3	rs17810546	161147744	G	0.16	0.022	CeD	G	1.36	$4x10^{-28}$	*IL12A*	19
10	rs1250552	80728033	G	0.44	0.020	CeD	NR	1.12	$9x10^{-10}$	*ZMIZ1*	19
2	rs3087243	204447164	G	0.58	0.001	T1D - CeD	A	NR	$8x10^{-11}$	*CTLA4* *SH2B3 - LNK*	20,22
12	rs17696736	110971201	G	0.47	0.036	T1D - CeD	G	1.34	$2x10^{-14}$	*TRAFD1 - PTPN11*	20
1	rs2476601	114179091	A	0.12	$2.4x10^{-9}$	T1D	T	1.98	$2x10^{-80}$	*PTPN22*	20
10	rs61839660	6134703	T	0.09	0.001	T1D	NR	1.6	$5x10^{-09}$	*IL2RA*	20
11	rs1004446	2126719	C	0.61	$1.2x10^{-7}$	T1D	C	1.61	$4x10^{-09}$	*INS*	22
11	rs7111341	2169742	T	0.27	$4.6x10^{-4}$	T1D	NR	NR	$4x10^{-48}$	*INS*	20
12	rs11171739	54756892	C	0.43	0.026	T1D	C	1.34	$1x10^{-11}$	*ERBB3*	33
16	rs12708716	11087374	A	0.64	0.034	T1D	G/A	NR	$7x10^{-13}$	*CLEC16A /KIAA0350*	22
16	rs4788084	28447349	G	0.57	0.049	T1D	G	1.09	$3x10^{-13}$	*IL27*	20

Table 3: Association of previously described variants for CeD and T1D in the dataset double autoimmunity versus patients with T1D only. A1, allele associated; Allele freq., allele frequency for which OR is reported; Chr, chromosome; Gene reported, the most plausible gene reported by the literature; NR, not reported; Position in base pair; Ref, reference

Chr	SNP	Position	A1	Allele freq.	OR [CI 95%]	*p* value	Reported Disease	Risk Allele	OR reported	*p* value reported	Gene reported	Ref
1	rs2816316	190803436	A	0.83	1.32 [1.15, 1.53]	0.03086	CeD	A	1.25	$2x10^{-17}$	*RGS1*	22
2	rs4675374	204510823	A	0.23	1.33 [1.08, 1.64]	0.00728	CeD	A	1.14	$6x10^{-09}$	*CTLA4*, *ICOS*, *CD28*	22
3	rs11712165	120601486	C	0.38	1.22 [1.01, 1.46]	0.03101	CeD	C	1.13	$8x10^{-09}$	*CD80*, *KTELC1*	22
3	rs1464510	189595248	A	0.46	1.28 [1.07, 1.54]	0.006945	CeD	A	1.29	$3x10^{-40}$	*LPP*	22
2	rs3087243	204447164	G	0.61	1.36 [1.23, 1.51]	0.00126	T1D + CeD	G	1.15	$8x10^{-11}$	*CTLA4*	23,31
10	rs12251307	6163501	T	0.09	1.46 [1.06, 2.0]	0.01756	T1D	T	NR	$1x10^{-13}$	*IL2RA*	23

Table 4: Haplotype and genotype HLA association and frequency comparison between healthy control subjects and patients with double autoimmunity,T1D only, or CeD only. Freq, frequency; OR, odd's ratio

		T1D+CeD/CeD				T1D+CeD/T1D			
Haplotype	Freq. Controls	Freq. T1D+CeD	Freq. CeD-only	OR [CI 95%]	*P*	Freq. T1D+CeD	Freq. T1D-only	OR [CI 95%]	*P*
DQ2.5	0.14	0.520	0.446	1.035 [0.860, 1.249]	0.972	0.446	0.318	1.442 [1.189, 1.748]	0.0003
DQ2.2	0.094	0.047	0.155	0.255 [0.173, 0.374]	0.422	0.046	0.040	1.201 [0.793, 1.821]	0.381
DQ8	0.1	0.350	0.064	5.086 [3.883 6.662]	0.163	0.346	0.392	0.939 [0.779, 1.131]	0.520
Other	0.663	0.157	0.260	0.467 [0.366, 0.595]	0.500	0.163	0.249	0.660 [0.530, 0.821]	0.0001
Genotype									
DQ2.5/DQ2.5	0.020	0.176	0.192	0.99 [0.96, 1.02]	0.914	0.168	0.066	1.20 [1.14, 1.26]	0.005
DQ2.5/DQ2.2	0.032	0.039	0.232	0.84 [0.82, 0.87]	7.29E-4	0.039	0.017	1.16 [1.06, 1.27]	0.242
DQ2.5/DQ8	0.027	0.350	0.067	1.47 [1.41, 1.53]	3.31E-10	0.350	0.377	0.98 [095, 1.01]	0.681
DQ2.5/Other	0.184	0.150	0.357	0.87 [0.87, 0.90]	1.9E-3	0.168	0.112	1.07 [1.03, 1.11]	0.688
DQ2.2/DQ2.2	0.012	0.005	0.004	1.03 [0.83, 1.28]	0.905	0.004	0.002	1.18 [0.88, 1.58]	0.169
DQ2.2/DQ8	0.022	0.035	0.012	1.22 [1.10, 1.36]	0.189	0.033	0.036	0.98 [0.92, 1.06]	0.908
DQ2.2/Other	0.111	0.012	0.059	0.87 [0.82, 0.92]	0.129	0.010	0.025	0.91 [0.83, 0.99]	0.326
DQ8/DQ8	0.009	0.083	0.010	1.6 [1.46, 1.74]	4.2E-4	0.083	0.078	1.00 [0.96, 1.05]	0.886
DQ8/Other	0.135	0.148	0.031	1.40 [1.32, 1.49]	1.46E-4	0.143	0.216	0.94 [0.91, 0.97]	0.175
Other/Other	0.449	0.002	0.036	0.85 [0.79, 0.92]	0.166	0.002	0.072	0.84 [0.80, 0.89]	0.028

Discussion:

It is possible that a subgroup of patients with T1D or CeD have certain characteristics that predispose them to develop both diseases. However, the larger percentage of individuals developing double autoimmunity than expected based on the prevalence of the individual diseases suggests that common genetic loci and common biological pathways are involved in the pathogenesis of double autoimmunity. By comparing the T1D and CeD GWAS results, we analyzed 12 shared genetic loci both within and outside the MHC-HLA region.

Targeted screening for CeD is recommended in high-risk groups such as children with T1D[27]. Screening for CeD in children is recommended as soon as they develop T1D, and, in the case of a negative outcome, this test should be repeated at well-defined intervals for at least 10 years[10,39]. Untreated CeD carries the risks of iron deficiency anaemia, growth retardation, osteoporosis, neuropsychiatric disorders, fertility problems, and gastrointestinal malignancies such as intestinal lymphoma. Genetic risk profiling can contribute to identifying patients with T1D who are predisposed to develop CeD and who might benefit from closer monitoring, as in the majority of cases (>90%), the diagnosis of T1D precedes that of CeD.

Our aim in the T1DGC Autoantibody Workshop was to enhance the understanding of why a single patient develops two autoimmune diseases by investigating the associated genetic risk factors. In the future, this information might also aid in building genetic risk models to identify individuals with either T1D or CeD who are at high risk of developing double autoimmunity. In our analysis, the HLA locus still presents the most important association with double autoimmunity. However, our association study shows that the HLA haplotypes or genotypes that are related with the risk of double autoimmunity are not the same as those related to the risk of either T1D or CeD in isolation. Individuals with both diseases more closely resemble the patients with T1D only with respect to the frequency of the *DQ2.5/DQ8* genotype, which is a well-known risk combination for T1D. The group of *DQ2.5/DQ8* carriers is infrequent in the general population (about 2.5%), yet these individuals have a more than fivefold increased risk of developing either T1D or double autoimmunity. Thus, the periodic screening of T1D-related autoantibodies in predominantly CeD patients carrying *DQ2.5/DQ8* could be helpful for identifying T1D at an early stage

of the disease. The same approach applies to patients with T1D carrying *DQ2.5/DQ2.5*, who should be screened for CeD antibodies.

We did not observe a significant enrichment of the shared risk alleles in the group of double autoimmunity patients. In our analysis, we did observe a similar number of CeD-only or T1D-only loci for both study groups. We are aware of the lack of follow-up of the patients but, based on epidemiology, would not have expected a significant increase in the number of unnoticed double autoimmunity patients that could modify the results[40]. We did not find any proof for our hypothesis that known shared genetic risk factors contribute to the coexistence of multiple diseases in the same individual. Nevertheless, *CTLA4* has been associated with multiple autoimmune diseases and has a well-known role in the activation, differentiation, and proliferation of T cells[41]. In our analysis, the *CTLA4* SNP rs3087243 showed a significant association with double autoimmunity in both data sets. While this SNP has not been associated with CeD in GWAS reports, it is in linkage disequilibrium (r^2 = 0.144; D' = 0.86) with rs4675374, which is associated with CeD risk[19]. These data suggest that *CTLA4* can contribute to the development of double autoimmunity. We also observed the significant association of SNPs located in the *IL2RA* locus. The functional role of *IL2RA* is highly related to *CTLA4,* with a possibly synergistic role, for example, in regulating the activation and differentiation of CD4-positive T cells[42].

In conclusion, we have shown that there are different genetic associations between patients with double autoimmunity, T1D only, or CeD only. The impact of genetic risk is based, primarily, on specific alleles and genotypes in the HLA class II region, with some support for two genes (*CTLA4* and *IL2RA*) that may be linked through a common immune pathway. The HLA and non-HLA loci found in this study can be used as stratification factors in the construction of risk models to predict double autoimmunity and for pathway enrichment analysis to enhance our understanding of the pathophysiology involved in the development of double autoimmunity. It should be noted that our analysis only included individuals of Caucasian origin. Hence, populations with other genetic backgrounds should be carefully checked, as the results may differ owing to differences in genetic background. The question of how these genetic factors influence the development of double autoimmunity requires further study.

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Chapter 7

Screening for coeliac disease in adult patients with type 1 diabetes mellitus: myths, facts and controversy

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Abstract

This review aims at summarizing the present knowledge on the clinical consequences of concomitant coeliac disease (CD) in adult patients with type 1 diabetes mellitus (T1DM). The cause of the increased prevalence of CD in T1DM patients is a combination of genetic and environmental factors. Current screening guidelines for CD in adult T1DM patients are not uniform. Based on the current evidence of effects of CD on bone mineral density, diabetic complications, quality of life, morbidity and mortality in patients with T1DM, we advise periodic screening for CD in adult T1DM patients to prevent delay in CD diagnosis and subsequent CD and/or T1DM related complications.

Introduction:

Coeliac disease (CD) is a permanent intolerance to ingested gluten resulting in immune mediated inflammatory damage to the small intestinal mucosa and a subsequent malabsorption syndrome[1]. Diagnosis of CD requires duodenal biopsy when the patient is on a gluten-containing diet and for the vast majority of adult patients also positive serology[2]. CD is one of the commonest lifelong disorders encountered in Western countries with a prevalence of about 0.6 % in the general population[3] and is, in particular in genetically susceptible individuals, associated with other autoimmune disorders including type 1 diabetes mellitus (T1DM) and autoimmune thyroiditis[4]. T1DM is characterized by T-cell mediated destruction of the insulin-producing ß-cells in the pancreas leading to hyperglycaemia and diabetic ketoacidosis[5]. Diabetes is diagnosed based on 1) plasma glucose criteria, either the fasting plasma glucose (FPG) or the 2-h plasma glucose (2-h PG) value after a 75-g oral glucose tolerance test (OGTT) or 2) on a glycated haemoglobin (HbA1c) value of > 6.5%[6]. Long term diabetic complications consist of micro- and macrovascular disease, which account for the major morbidity and mortality associated with T1DM[7]. Up to one third of patients with T1DM have thyroid antibodies, and half of these patients may progress to clinical autoimmune thyroid disease[8]. The need for annual screening for thyroid disease in T1DM patients has therefore been recommended.
The over all prevalence of CD in T1DM patients is about 6%[9]. The association between CD and T1DM was first noted over 40 years ago in children[10]. Therefore, screening in paediatric T1DM patients is advocated. However, international paediatric consensus based guidelines differ in the need and frequency of screening for CD[11]. Some recommend an annual screening interval by testing antibodies against tissue transglutaminase 2 (TG2A), others advice to perform these tests in the presence of typical CD symptoms only[11]. However, despite the high prevalence of CD in T1DM patients there is no consensus on screening adult T1DM patients for CD.
In this review it is discussed whether screening for CD should be performed in adult T1DM patients and at which interval. For this purpose, the current literature was screened with respect to the clinical features of patients with both diseases as compared to patients with T1DM alone.

Association between CD and T1DM:

Genetics

T1DM and CD are auto-immune, inflammatory diseases for which the major genetic contribution arises from the major histocompatibility complex[12]. These so-called HLA-DQ heterodimers enable the presentation of peptides that are derived from otherwise innocuous self- or non-self antigens (proteins from insulin producing beta cells in T1DM, gliadins in CD) and activate pathogenic effector T-cells [13]. Besides the genetic overlap in the major histocompatibility complex, genome wide association studies (GWAS) in these two diseases have revealed a large number of well validated, non-HLA genetic risk loci providing an opportunity to explore the possibility of overlapping susceptibility between them[12]. Thus, genetic overlap exists between CD and T1DM consisting of both HLA and non-HLA genes[14-16]. Both disorders are associated with the major histocompatibility complex (MHC) class 2 antigen DQ encoded by the alleles DQA1*05 with DQB1*02 (DQ2.5) and DQA1*03 with DQB1*03:02 (DQ8)[1,17].

In patients with CD, individuals who are HLA-DQ 2.5 homozygous have a greater risk of developing CD and the gluten specific T-cell response is more vigorous when gluten peptides are presented by antigen presenting cells homozygous for HLA-DQ 2.5[18,19]. In European Caucasian populations, more than 90% of CD patients carry the HLA-DQ 2.5 heterodimer and the majority of CD patients who do not carry this HLA-DQ 2,5 heterodimer are HLA-DQ8 or HLA-DQ2.2 positive[20].

The main determinant of risk of developing T1DM is HLA-DQ8 and to a lesser extent HLA-DQ 2.5[21,22]. In a recent study, we compared the frequency of HLA-DQ haplotypes between 2472 T1DM patients versus 483 T1DM + CD patients[16]. In patients with T1DM, the HLA-DQ 2.5 haplotype showed a significant association and provided the highest risk for developing double autoimmunity (OR = 1.44, p-value = 0.0003, Table 1). As expected, the absence of the haplotypes HLA-DQ 2.5, DQ8 and DQ 2.2 (which is classified as "other" which is present in about 25% of T1DM patients), showed the strongest protection (OR= 0.66, P=0.0001, Table 1). Therefore, an HLA-DQ 2.5 negative T1DM patient does not require monitoring for CD.

Table 1. Haplotype and genotype HLA association and frequency comparison between double autoimmunity versus type 1 diabetes-only[16].

		T1DM + CD versus T1DM			
Haplotype	Frequency controls	Frequency T1DM + CD	Frequency T1DM only	OR (CI 95%)	P value
DQ 2.5	0.14	0.446	0.318	1.442 (1.189, 1.748)	0.0003
DQ 2.2	0.094	0.046	0.040	1.201 (0.793, 1.821)	0.381
DQ8	0.1	0.346	0.392	0.939 (0.779, 1.131)	0.520
Other	0.663	0.163	0.249	0.660 (0.530, 0.821)	0.0001
Genotype					
DQ 2.5/ DQ 2.5	0.020	0.168	0.066	1.20 (1.14, 1.26)	0.0005
DQ 2.5/ DQ 2.2	0.032	0.039	0.017	1.16 (1.06, 1.27)	0.242
DQ 2.5/ DQ8	0.027	0.350	0.377	0.98 (0.95, 1.01)	0.681
DQ 2.5/ other	0.184	0.168	0.112	1.07 (1.03, 1.11)	0.688
DQ 2.2/ DQ 2.2	0.012	0.004	0.002	1.18 (0.88, 1.58)	0.169
DQ 2.2/ DQ 8	0.022	0.033	0.036	0.98 (0.92, 1.06)	0.908
DQ 2.2/ Other	0.111	0.010	0.025	0.91 (0.83, 0.99)	0.326
DQ 8/ DQ 8	0.009	0.083	0.078	1.00 (0.96, 1.05)	0.886
DQ 8/ Other	0.135	0.143	0.216	0.94 (0.91, 0.97)	0.175
Other/ Other	0.449	0.002	0.072	0.84 (0.80, 0.89)	0.028

Abbreviations: CD= coeliac disease, OR= Odd's ratio, T1DM= type 1 diabetes mellitus.

In addition to the overlap between T1DM and CD in HLA genes, it was revealed that non-HLA genes overlap as well[12,16]. *CTLA-4* and *IL2RA* loci are more strongly associated with double autoimmunity than with either T1DM or CD alone[16]. The combination of HLA and non-HLA variants might improve risk prediction for potential CD[23].

Environmental factors

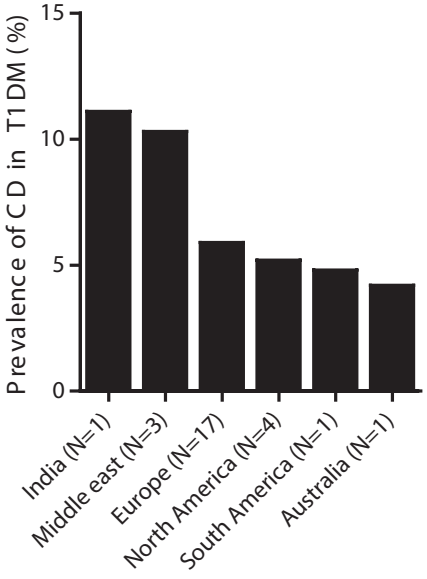
Several environmental factors have been investigated as precipitating factors for the development of T1DM or CD. A popular theory, based on possible molecular mimicry, is the association between autoimmune diseases and viral infections. Prime viral candidates that have been shown to cause precipitation to T1DM are enteroviruses, more specifically Coxsackie viruses[24]. Moreover, rotavirus infection increases the risk for developing T1DM and an association between rotavirus and increased risk for CD has been described as well[25,26]. Furthermore, an altered composition of bacteria in the gut, altered gut permeability and intestinal inflammation seem to be factors that contribute to the development of T1DM[27]. Exposure to cereals has been described as a risk factor for the development of both T1DM and CD related autoantibodies. However, these studies show conflicting results[28-30].

Demographic characteristics:

Epidemiology

Many studies have investigated the prevalence of CD in paediatric and adult T1DM patients by different serological screening methods (gliadin, anti endomysium (EMA), anti tissue transglutaminase (TG2A) and anti reticulin antibodies). The prevalence of CD in T1DM patients (children and/or adults) is reported to vary between 0.8% and 16.4% with a mean prevalence of 6%[4,9,31]. A large meta-analysis identified 27 studies, which included in total 26 605 individuals with T1DM[9]. Seventeen studies were performed in Europe, 4 in North America, 1 in South America, 1 in Australia, 3 in the Middle East and 1 in India (Figure 1)[9]. A remarkable high prevalence of CD in T1DM patients is seen in studies performed in Algeria (16.4%), India (11.1%) and Saudi Arabia (11.3%)[32-34]. The relatively high frequency of HLA-DQ 2.5 in the Middle East and India possibly contributes to the high prevalence of CD in T1DM[35]. Furthermore, these countries have a per capita wheat consumption that ranks among the highest in the world[35]. This high prevalence still needs to be confirmed in additional studies. Data from East-Asian and African T1DM cohorts and CD screening are lacking in current literature.

Figure 1: Mean prevalence of screen detected coeliac disease (CD) in children and adults with type 1 diabetes mellitus (T1DM) around the world. Mean prevalence is calculated from studies with at least 100 patients with T1DM[9]. N indicates the number of screening studies performed on each continent.



Clinical presentation

The clinical presentation of CD in T1DM patients resembles that in non-T1DM patients and consists of gastrointestinal complaints (diarrhoea, constipation, vomiting, abdominal distension, anorexia) or extra-intestinal complaints such as growth failure, anaemia, decreased bone mass or osteoporosis, and dental enamel defects[4]. However, CD patients might also be asymptomatic and may have subtle complaints indicative of CD and may only be recognized in retrospect following the benefits of a GFD[36] . Previous studies have reported that 45% to 60% of patients with T1DM and CD did not have any complaints of CD indicating a diagnostic challenge[37,38].

Furthermore, gastrointestinal complaints are common in T1DM patients and a broad differential diagnosis exists for these patients (Table 2)[39,40]. Furthermore, the fact that a large part of patients presents only with mild symptoms or seem to be asymptomatic provides difficulties for detecting CD [41]. It has been demonstrated that the risk of CD in T1DM patients is associated with age of onset of T1DM. Children with age of onset of T1DM younger than 4 years are at higher risk to develop CD than those with older age of onset[42]. Regarding clinical practice, we observed two peaks in the age of CD diagnosis in T1DM patients: around 10 and 45 years of age[41]. T1DM diagnosis precedes CD diagnosis in about 90% of patients and females with T1DM have a higher risk of the additional diagnosis of CD than males[41,42].

A new syndrome of gluten intolerance, non coeliac gluten sensitivity (NCGS), has been described. NCGS can be diagnosed in those patients with gluten intolerance who do not develop antibodies that are typical neither of CD nor of wheat allergy and who do not suffer from lesions in the duodenal mucosa[43].

Table 2: Differential diagnosis of gastrointestinal complaints in T1DM patients[39,40,105,106].

Causes of gastrointestinal complaints in T1DM patients
Coeliac disease
Diabetic gastropathy
Gastroesophageal reflux disease
Mesenteric ischemia
Irritable bowel syndrome
Hyperglycaemia affects GI motor function and perceptions of the GI tract
Metformin use
Depression
Eating disorders

Although disease characteristics of NCGS are overlapping with irritable bowel syndrome (IBS), a recent study observed that an associated autoimmune disease was present in 14% of patients with NCGS, which was mainly autoimmune thyroiditis and sporadically T1DM[44].

Adherence to a GFD

Nutrition therapy is an important issue in the management of T1DM and the cornerstone of treatment in patients with CD[6,45] . In T1DM, dietary interventions aim to maintain blood glucose, blood pressure, lipid levels and body mass index in the normal range[46]. A GFD together with an insulin therapy integrated into an individual's dietary and physical activity pattern imposes practical limitations and leads to restrictions in the lifestyle of a child or adolescent. Therefore, it may not be surprising that non adherence to a GFD in T1DM patients with CD is more common than in CD patients[47,48] . Another problem that arises is the availability of gluten free food. In 5 different US states it was found to be significantly less available than food containing gluten[49]. The increased cost of GFD products may have an impact on compliance in T1DM patients with CD as well[49]. Therefore, we advise that patients with both conditions are guided by a skilled dietitian.

Clinical consequences of CD in adult patients with T1DM:

So far, studies addressing the consequences of CD in adult T1DM patients differ in methodology, study size and prospective/retrospective design. Therefore, these results are difficult to compare and interpret. An overview of these results is given in Table 3.

Glycaemic control

In adult patients with T1DM, no significant change of HbA1c levels was found, when comparing before CD diagnosis, at CD diagnosis and after treatment of CD by a GFD[50,51]. This data is confirmed in a recent population based cohort study which found that having a diagnosis of CD does not influence the risk of hospital admission due to hypoglycaemia, keto-acidosis or coma in T1DM patients[52].

Lipid profile

Undetected CD in the general population is associated with lower cholesterol levels, which is thought to contribute to a favourable cardiovascular risk profile in untreated CD patients[53]. Accordingly, lower levels of cholesterol and triglycerides

were found in newly detected, untreated CD patients with T1DM[54]. The assumed mechanism that may contribute to the lower cholesterol levels in undetected CD patients is malabsorption.

Microvascular complications

Intensive insulin therapy to normalize blood glucose levels effectively delays the onset and slows the progression of microvascular complications including diabetic retinopathy, nephropathy and neuropathy in T1DM patients[55-57] . Several studies investigated the influence of (newly diagnosed) CD with or without treatment by a GFD on long term diabetic complications and found CD to be either protective[51,54,58] or aggravating[59-61]. A recent large nationwide study in Sweden revealed that the duration of CD is important for the eventual effect[60]. They showed that individuals with T1DM and CD were at a lower risk of diabetic retinopathy in the first 5 years after CD diagnosis (adjusted hazard ratio (HR) 0.57 [95% CI 0.36-0.91]), followed by a neutral risk in years 5 to <10 years (1.03 [0.68-1.57]). With longer follow-up, coexisting CD was a risk factor for diabetic retinopathy (10 to <15 years of follow-up, adjusted HR 2.83 [95% CI 1.95-4.11]; ≥15 years of follow-up, 3.01 [1.43-6.32])[60]. They ascribe the protective effect in the first 5 years to lower cholesterol levels and lower body mass index (BMI). However, this study lacks individual-based information on GFD adherence.

In a study of our group we found less diabetic retinopathy in a T1DM population with a mean CD duration of 3 years + treatment by GFD compared to T1DM patients without CD[51]. Also, a previous study by Pitocco et al showed more subclinical atherosclerosis in T1DM patients with a mean duration of treated CD of 9.9 years[61]. These studies suggest that a short duration of CD is protective and a longer duration of CD may aggravate diabetic complications[51,60].

Renal disease

CD is associated with a higher risk of end-stage renal disease (ESRD) with a Hazard Ratio (HR) for ESRD of 2.87 (95% CI 2.22 to 3.71, $p<0.001$)[62]. The cumulative prevalence of end-stage renal disease in T1DM patients without CD, is 2,2% at 20 years and 7,7% at 30 years[63]. Interestingly, in T1DM patients with CD it was found that non-adherence to a GFD was associated with early elevation of albumin excretion in urine, a recognized factor for diabetic nephropathy[64]. Skovbjerg et al found that there was a higher prevalence of CD in T1DM patients with nephropathy (2,6%) than in T1DM patients without nephropathy (1%)[65]. A recent study found a positive

association between longstanding CD in T1DM patients and chronic renal disease in T1DM[66]. For chronic renal disease, this excess risk was present after more than 10 years of CD (HR 2.03, 95% CI 1.08, 3.79)[66]. However, data about GFD adherence was lacking. These studies suggest that concomitant CD in T1DM patients might lead to more nephropathy in case of longstanding CD, in particular in case of poor adherence to a GFD[64]. The underlying mechanisms need, however, to be elucidated.

Bone mineral density

Decreased bone mineral density (BMD) is observed both in T1DM patients[67] and in CD patients[68]. In the latter group of patients, this is especially related to the intestinal malabsorption of vitamin D, necessary for healthy bone metabolism[68]. Reports have shown that bone mineral density is lower in paediatric T1DM patients with undiagnosed CD than in T1DM patients without CD[69,70]. As expected, also in adults with both T1DM and active CD, a decreased BMD was found, but whether CD or T1DM was the cause remains unclear[71]. A study by Sategna-Guidetti showed that treatment by a GFD results in an improvement of lumbar spine BMD in adults with CD[72].

In summary, BMD in T1DM + CD patients is generally decreased and follow-up of BMD with possible treatment is warranted. Besides maintaining a GFD, data is scarce whether calcium and vitamin D supplementation in CD patients is mandatory[68]. Lifestyle changes as regular exercise and smoking cessation should be advised, and in the case of osteoporosis, calcium, vitamin D and bisphosphonates should be prescribed[68].

Quality of life

Both T1DM and CD are chronic illnesses which influence the quality of life (QOL) since the treatments are burdensome and the complications can be debilitating and life threatening. T1DM patients have a diminished QOL which is partly caused by the development of vascular complications[73]. The lower QOL in CD patients is reported especially in the social aspects of life and in those with symptoms, women being mostly affected[74]. In adult T1DM patients with both T1DM and treated CD, we described a compromised QOL particularly in women and both social functioning and general health perception was affected[75]. This is of importance since patients with T1DM are at increased risk of depression[76]. The additional diagnosis of CD further increases the risk of depression, and this should be taken into account in the clinical support of these patients[77].

Comorbidity and mortality

T1DM is, beside CD, associated with autoimmune thyroid diseases (Hashimoto's or Graves' disease) (AIT), autoimmune gastritis, Addison's disease, and vitiligo[8]. The presence of a third autoimmune disease in T1DM + CD patients is frequently found. A study by Kaspers et al. found a higher incidence of AIT in patients with T1DM and CD (6.3%) when compared to those with CD alone (2.3%)[78]. Our clinical practice study in adults revealed that 28% of T1DM + CD patients were diagnosed with a third autoimmune disease, mainly autoimmune thyroiditis (22%)[79].

A small group of patients with CD fail to improve clinically and histologically upon elimination of dietary gluten and this complication is referred to as refractory coeliac disease (RCD)[80]. RCD imposes a serious risk of developing enteropathy-associated T-cell lymphoma (EATL). The prevalence of RCD and EATL in the general population is very rare and studies investigating the risk of developing RCD or malignancy in T1DM + CD patients are currently lacking[81].

The question whether CD influences the mortality in T1DM patients was recently investigated in Sweden[82]. These authors described that having a CD diagnosis for more than 15 years was associated with a 2.8-fold increased risk of death in individuals with T1DM[82]. They hypothesized that the excess mortality was caused by persistent low grade inflammation due to CD or poor adherence to a GFD while using insulin therapy.

Rationale for screening for CD in adult T1DM patients:

CD fulfills many of the WHO criteria for screening in patients with T1DM but not all of them[83]. CD is common and well defined, screening tests are simple + safe + accurate, screening seems to be culturally acceptable, treatment is available and clinical detection of CD can be difficult. However, studies are lacking whether screening for CD in T1DM patients is cost effective and it is currently unknown whether screen detected asymptomatic CD patients benefit from starting with a GFD. The latter will be investigated by the CD-DIET study[84] which is designed as a prospective controlled trial in which asymptomatic screen detected CD patients will be treated with or without a GFD. The results of the efficacy and safety of a GFD in patients with T1DM with asymptomatic CD will add significant data to the discussion about screening for CD in T1DM patients[84].

Table 3. Clinical consequences of coeliac disease (CD) in adult Type 1 Diabetes Mellitus (T1DM) patients as compared to T1DM without CD. ? = no studies performed. NA = not applicable.

Clinical consequence	T1DM + CD	Patients on GFD	References
HbA1c	Hba1c in screen detected CD patients is lower (Kaukinen, Bakker), higher (Leeds).	NA	Kaukinen, 1999
		NA	Bakker, 2013
		NA	Leeds, 2011
	No difference in HbA1c during follow up (Kaukinen, Bakker)	Yes	Bakker, 2013
		Yes	Picarelli, 2013
	No increased risk for hospital admission due to hypoglycemia, keto-acidosis or coma	Unknown	Kurien, 2015
Cholesterol + triglycerides	Lower in screen detected CD patients	NA	Leeds, 2011
		NA	Picarelli, 2013
Nephropathy	Higher prevalence of nephropathy	Unknown	Skovbjerg, 2005
		Unknown	Mollazadegan, 2014
Retinopathy	< 10 years of CD results in less retinopathy, more than 10 years	Unknown	Mollazadegan 2012
	leads to more retinopathy	Yes	Bakker, 2013
Bone mineral density	Lower BMD at diagnosis	NA	Lunt , 2001
Quality of life	Decrease, particularly in women, both social functioning and general health perception are affected	Yes	Bakker 2013
Depression	Increased risk	Unknown	Garud, 2009
Refractory Coeliac disease	?		?
Enteropathy associated T cell lymphoma	?		?
Mortality	A diagnosis of CD for > 15 years increases the risk of death in patients with T1D.	Unknown	Mollazadegan 2013

Consequently, there is still no consensus on screening adult patients with T1DM for CD. International guidelines for adult CD and T1DM differ in their recommendations for screening of CD in T1DM patients[2,6,85-91] (Table 4). At present, a case-finding approach in adult T1DM patients is most acceptable, ethically and financially[2,92]. However, a recent study in the United States and Canada underscores the need for an uniform screening program. This study revealed a high variability in testing for CD in T1DM patients together with an inconsistency of management of CD[93]. In addition, we have recently reported that approximately 20% of patients with T1DM and CD reported to have had CD related complaints for at least 5 years before CD diagnosis was made[79]. The long term consequences of a diagnostic delay are currently unknown. The high prevalence of several complications as reported in Table 3 in T1DM + CD patients, together with improvement of BMD after start of a GFD provides a strong rationale for an uniform screening program together with careful monitoring. Further, a recent randomized study showed that screen-detected and apparently asymptomatic EmA-positive patients at risk for CD benefit from a GFD as measured by extensive clinical, serologic, and histologic parameters[94]. Hypothetically, this data might be extrapolated to asymptomatic CD in T1DM patients. Another argument for screening is the fact that the incidence of T1DM and CD is rising over time[95,96].

Table 4: Clinical recommendations for screening of CD in T1DM patients in adult CD and T1DM guidelines. BMI = Body Mass Index, CD = Coeliac Disease, T1DM = Type 1 Diabetes Mellitus

Guidelines	Year	Recommendation	Reference
CD guidelines			
Gastroenterological Society of Australia	2007	Not reported	85
Dutch Society of Gastroenterology	2008	Testing for CD in case of clinical suspicion.	86
World Gastroenterology Organisation	2013	Not reported.	88
American College of Gastroenterology	2013	Testing for CD if there are any digestive symptoms, or signs, or laboratory evidence suggestive of CD.	89
British Society of Gastroenterology	2014	Testing for CD should be performed when CD is suspected.	2
National Institute for Health and Care Excellence (NICE)	2015	Test for CD at the moment of CD diagnosis and in case of persisting symptoms.	90
T1DM guidelines			
American Diabetes Association	2014	Screening for CD soon after T1DM diagnosis, thereafter screening should be considered based on signs and symptoms.	6
National Institute for Health and Care Excellence (NICE)	2015	In case of low BMI or weight loss, screen for CD.	87
Australian Diabetes Society	2011	Screen for CD at diagnosis and at least in the first fiver years after diagnosis.	91

We propose the following screening algorithm (Figure 2) for CD in adult T1DM patients. CD should be diagnosed by serology and duodenal biopsy with the patient on a gluten-containing diet[2]. Serology is by TG2A and if patients are IgA deficient, IgG-TG2A can be used. Villous atrophy (Marsh IIIa- IIIc) is required for diagnosis of CD[2]. Due to the high sensitivity and specificity of TG2A, this test is used for

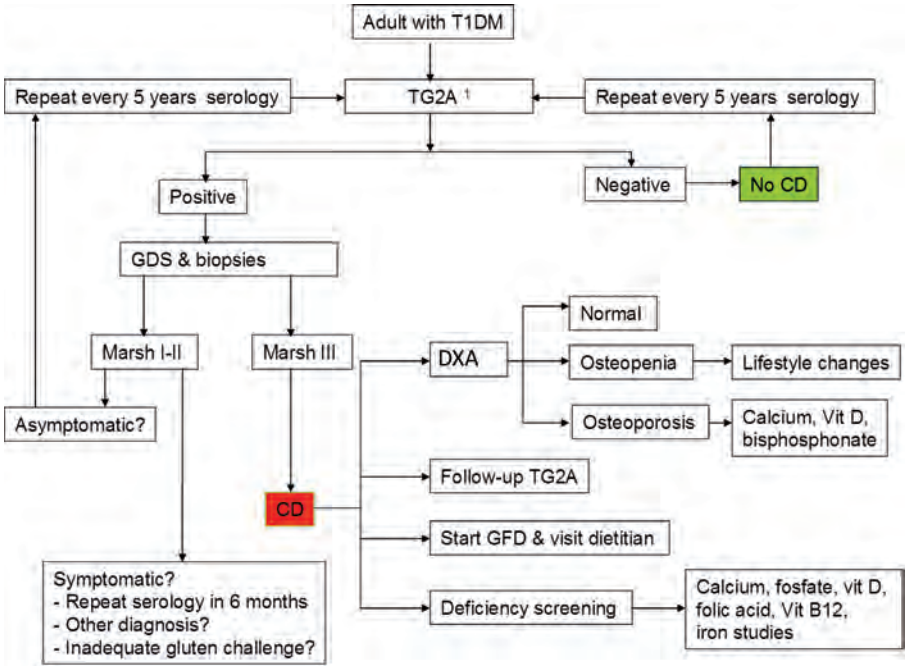
screening in T1DM patients[97]. In case of IgA deficient individuals, or in patients with high probability of CD, IgG TG2A should be tested as 2% of CD patients are IgA deficient[2] . As T1DM patients might have transient elevations of TG2A, a confirmatory small intestinal biopsy is recommended[98,99]. In case of a biopsy with Marsh I-II, a serological repetition in 5 year is recommended. Further, another differential diagnosis for intraepithelial lymphocytosis should be considered (e.g. giardia, Olmesartan induced, small intestinal bacterial overgrowth). So far, only retrospective data is available and prospective studies are needed to determine a screening interval for CD in T1DM patients. As proposed by DeMelo et al[100], we suggest to repeat TG2A testing every 5 years in case of negative serology. A recent systematic review found that most cases of CD are diagnosed within 5 years of T1D diagnosis and they advise screening at T1DM diagnosis and within 2 and 5 years thereafter[101]. Only the Australian Diabetes Society recommends screening for CD after 5 years of T1DM diagnosis (Table 4). As studies are lacking investigating the screening frequency in T1DM patients, we advocate continuing screening every 5 years for CD in T1DM patients. In the presence of CD a clinical work-up should be performed to evaluate and possibly treat bone mineral density and vitamin deficiencies (Figure 2). Based on current data, this screening algorithm is not applicable to all countries as studies about prevalence of CD in T1DM patients are lacking from several countries (Figure 1).

HLA-DQ typing

The European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines recommend assessing the HLA–DQ2.5/DQ8 genotype in patients with T1DM, as an initial approach for CD screening. A recent study investigated the clinical relevance and cost-effectiveness of human leukocyte antigen (HLA)-genotyping in T1DM patients as a screening tool[102]. They found that HLA-DQ typing in T1DM patients is neither distinctive nor cost-effective in screening for CD[102]. This might be due to the fact that only 25% of T1DM patients is HLA-DQ 2.5 or DQ 8 negative[14,16]. Thus, in our algorithm HLA-DQ typing is excluded.

According to recent guidelines for symptomatic children who have high antibody titres, a duodenal biopsy is not needed anymore for diagnosing CD[103]. Indeed, a recent study showed that none of the T1DM children with high TG2A titres would have needed a biopsy for diagnosis[104]. Whether this is also the case in symptomatic adult T1DM patients with high TG2A titres remains to be established.

Figure 2: Proposed algorithm for the screening and follow-up of coeliac disease (CD) in asymptomatic patients with type 1 diabetes mellitus (T1DM).



DXA= Dual X-ray Absorptiometry, GFD= Gluten Free Diet, GDS= Gastroduodenoscopy
TG2A= Tissue transglutaminase 2 antibodies
1) IgA TG2A should be evaluated first, in IgA deficient individuals or in patients with high probability of CD IgG TG2A should be performed.

5. Conclusions

CD fulfills many of the WHO criteria for screening as it is common, simple to diagnose, and treatment is available. Detection of CD in T1DM patients is important as morbidity and mortality is increased in patients with both T1DM and CD. Furthermore, several clinical consequences are present in both disorders as decreased BMD, nephropathy, retinopathy and decreased QOL which need careful follow-up. We propose an algorithm for periodic screening and advise a multidisciplinary approach for these complex patients.

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Chapter 8

Summary and future perspectives

Summary

Coeliac disease is a permanent intolerance to ingested gluten resulting in immune mediated inflammatory damage to the small intestinal mucosa and a subsequent malabsorption syndrome in genetically predisposed individuals[1]. Gluten is a protein complex found in wheat, rye, and barley and treatment of coeliac disease consists of a gluten free diet. Coeliac disease can cause a wide variety of symptoms, including both intestinal (diarrhoea, weight loss, abdominal pain) and extra-intestinal (osteoporosis, anaemia)[1].

The association between coeliac disease and type 1 diabetes (T1DM) has long been established. T1DM is characterized by destruction of the insulin-producing β-cells in the pancreas leading to high blood glucose level outside the physiological range. The condition commonly produces classical symptoms of polydipsia (increased thirst), polyuria (frequent urination), increased fatigue and finally without treatment keto-acidotic coma. Treatment involves insulin therapy either in the form of multiple daily injections or pump therapy, together with calculated carbohydrate intake and frequent glucose monitoring[2]. As a result of chronic hyperglycaemia, a number of complications can occur in the long term, especially of microvascular origin including: diabetic retinopathy (eye damage), neuropathy (nerve damage), and nephropathy (kidney disease).

The prevalence of coeliac disease is rated 6 times greater in adults with T1DM than in the general population[3,4]. The increased prevalence of coeliac disease in T1DM patients is due to a common genetic background and interplay between environmental and immunological factors[5,6]. Both diseases have long term consequences, however, the additional long term consequences in case of presence of both disease is unknown.

This thesis concerns several clinical and genetic aspects of patients with both T1DM and coeliac disease. Further, we discuss whether screening for coeliac disease is indicated in patients with T1DM.

In **chapter 2** we evaluated common practice of diagnosing coeliac disease in T1DM patients in the Netherlands. We studied 118 patients with both T1DM and coeliac disease identified in the Netherlands and we retrospectively collected data

on sex distribution, age of onset of T1DM, age of coeliac disease diagnosis, type of complaints of coeliac disease, duration of complaints of coeliac disease before diagnosis, family history of coeliac disease or T1DM, comorbidity and HLA-DQ type. We observed a bimodal distribution of the age of diagnosis of coeliac disease in T1DM patients with a peak incidence at the age of 10 and 45 years. Furthermore, we found that a large proportion (48%) of our patients diagnosed with coeliac disease in adulthood reported to have had coeliac disease related complaints over 5 years before coeliac disease diagnosis was established. Our observation suggests that physicians should be more aware of the symptoms and/or the association of both diseases and that screening for coeliac disease is recommended in T1DM patients.

Patients with (undiagnosed) coeliac disease may have weight loss, diarrhoea, abdominal discomfort or osteoporosis, however, as indicated previously, data are sparse concerning the effect of concomitant coeliac disease in T1DM patients. In **chapter 3** we investigated the course of glycaemic control at coeliac disease diagnosis and after initiation of a gluten free diet (GFD) in T1DM patients and the prevalence of diabetic complications in T1DM patients with adult diagnosis of coeliac disease. We compared 31 patients with coeliac disease + T1DM with 46 T1DM patients matched for age, gender, T1DM duration and glycosylated haemoglobin A1c percentage (HbA1c levels). HbA1c is used as an marker for average plasma glucose concentrations over the past 2-3 months. We found that the diagnosis of coeliac disease and treatment thereafter with a GFD was not of significant influence on glycaemic control in T1DM patients. Further, we observed a lower prevalence of retinopathy in the T1DM + coeliac disease group compared with patients with T1DM only.

It was hypothesized that Advanced Glycation End products (AGEs) play a role in the lower prevalence of retinopathy in the concomitant presence of coeliac disease in T1DM patients[7] and this was investigated in **chapter 4.** AGEs are proteins or lipids that become glycated after exposure to sugars and AGEs are the result of the Maillard reaction. Although they may be formed as a result of normal metabolism and aging, their formation is exaggerated in the presence of certain pathologic conditions, e.g. oxidative stress due to hyperglycaemia in patients with diabetes. Not only are AGEs formed in certain pro-atherogenic conditions, also by the ambiguous presence of their receptors (RAGE) on the endothelium, AGEs have been shown to contribute

to the development of atherosclerosis[8,9]. Besides endogenously formed AGEs, it has been demonstrated that exogenously formed AGEs (dietary AGE's) are absorbed by the intestine into the bloodstream and represent a source of chemically active toxins[10]. A previous study from Australia observed lower levels of AGEs in patients with both T1DM and coeliac disease compared to T1DM alone, possibly because of a GFD low in dietary AGEs[7]. We therefore compared AGE levels between 25 patients with T1DM and coeliac disease, 25 T1DM patients without coeliac disease and 25 healthy controls. We measured AGE levels by skin autofluorescence (AF) and serum soluble receptor AGE (sRAGE). Although we could previously detect differences in the presence of microvascular complications, no differences were found in skin AF or sRAGE levels between T1DM patients with or without coeliac disease. We did observe higher skin AF levels in patients with T1DM compared to healthy controls. Therefore, our findings suggest that AGE levels are not responsible for the differences in microvascular complications between patients with T1DM with or without coeliac disease.

Since coeliac disease can only be treated by GFD that may differ completely from a regular diet taken by family and friends, coeliac disease might also affect the quality of life (QOL) in patients with T1DM. In **chapter 5** we compared QOL of 57 adult patients with both T1DM and coeliac disease with 57 T1DM patients matched for age, gender and socioeconomic status. Generic QOL scores were compared with data from healthy Dutch controls. In the group of patients with T1DM and coeliac disease, women had a lower score on the subscales social functioning, vitality and mental health compared to men. Comparing patients with T1DM + coeliac disease versus T1DM patients revealed that patients with T1DM + coeliac disease have more worries about social functioning and diabetes related complications. Comparison of patients with T1DM and coeliac disease versus healthy controls revealed that social functioning and general health perception is affected in patients with T1DM and coeliac disease. We therefore conclude that coeliac disease has an additional negative effect on quality of life in patients with T1DM which is an important aspect in the support, follow-up and treatment of these patients. We advocate that special attention should be addressed to this observation during out-patient contact with these patients.

In **chapter 6** we explored the genetic differences between individuals with both coeliac disease and T1DM versus those with only one disease. T1DM (n=42) and

coeliac disease (n=28) associated Single Nucleotide Polymorphisms (SNP's) and HLA haplotypes were compared in 543 subjects who developed T1DM and coeliac disease versus 2,472 patients with T1DM only and 2,223 coeliac disease only patients. Two association analyses were conducted: double autoimmunity versus T1DM and double autoimmunity versus coeliac disease. The CTLA4 and IL2RA loci were more strongly associated with double autoimmunity than with either T1DM or coeliac disease alone. The *HLA-DQ2.5* haplotype was significantly associated with double autoimmunity relative to T1DM only (OR 1.44, $P = 0.0003$). In clinical use, HLA-DQ typing might only be useful in excluding the possibility of developing coeliac disease in HLA-DQ 2.5 or 8 negative T1DM patients[6]. In addition, a Dutch study found that HLA-DQ typing in T1DM patients is neither distinctive nor cost-effective in screening for coeliac disease[11]. Our findings suggest that the impact of genetic risk is based, primarily, on specific alleles and genotypes in the HLA class II region, with some support for two genes (*CTLA4* and *IL2RA*) that may be linked through a common immune pathway. This information might aid in building genetic risk models to identify individuals with either T1DM or coeliac disease who are at high risk of developing double autoimmunity.

In the final chapter of this thesis (**chapter 7**) we provide a review of the present knowledge on the clinical consequences of concomitant coeliac disease in adult patients with T1DM and we discuss whether screening for coeliac disease is indicated in patients with T1DM. This overview shows that a delay in coeliac disease diagnosis is frequently found in T1DM patients and that coeliac disease in T1DM leads more often to a decreased bone mineral density (BMD), more diabetic complications, a decreased QOL and a higher mortality. We propose an algorithm for periodic screening (every 5 years) for coeliac disease in T1DM patients.
Screening is performed by testing antibodies against tissue transglutaminase 2 (TG2A) and in case of positivity a confirmatory small intestinal biopsy is recommended. If coeliac disease is diagnosed, a clinical work-up should be performed consisting of referral to a dietitian, initiation of a GFD, investigation of possible vitamin deficiencies and measurement of BMD. In case of negative TG2A serology should be repeated after 5 years. In case of gastrointestinal complaints with negative TG2A levels another diagnosis should be considered. An earlier diagnosis of coeliac disease might lead to less complications and a better QOL in T1DM patients.

Future perspectives

This thesis reveals novel insights into the clinical and genetic aspects of patients with both T1DM and coeliac disease, yet many areas require further study.

First, there are no effective strategies to prevent or cure autoimmune diseases. Both coeliac disease and T1DM have a strong HLA-association, indicative of the involvement of the adaptive immune system and the presence of autoantibodies is characteristic. In the affected individual with coeliac disease, 4 components interact: gluten, TG2A, HLA-DQ2/8 and T cells[12]. However, there is a lack of information on environmental factors that might trigger the autoimmune process. In coeliac disease, the HLA DQ association is very strong: approximately 95% of the patients express HLA-DQ2 and the remainder is mostly HLA-DQ8 positive[13] . Nevertheless, although some 40% of the population in the Western world expresses one or both of these HLA-DQ alleles, only 1% of the population develops CD. This suggests an environmental trigger in the pre-autoimmune process which is currently unknown. Alterations in the composition of the gut microbiota might play a role in coeliac disease development[14]. Studies report intestinal dysbiosis in coeliac disease patients; untreated and treated with a gluten-free diet (GFD), compared to healthy controls[15,16]. A GFD per se influences gut microbiota composition, and thus constitutes an inevitable confounding factor in studies conducted in CD patients. To improve our understanding of whether intestinal dysbiosis is the cause or consequence of coeliac disease, prospective studies in healthy infants at family risk of CD are needed[17]. In addition, studies have investigated several environmental factors for the development of T1DM (viral infections, lack of breast-feeding after birth and the consumption of cereals) although these factors are not strongly associated with T1DM. The microbiota might play a role since a recent study in humans suggests that the gastrointestinal microbiota tends to reach a more or less stable state proportionally with an infant's age, whereas children who developed β -cell immunity have a less diverse and stable gastrointestinal microbiota[18]. More prospective studies are needed to elucidate the microbiota as environmental trigger for both T1DM and coeliac disease.

Second, screening for CD in T1DM fulfils almost all of the WHO criteria for screening (Table 1). An important unanswered question is whether it is of benefit to diagnose and treat asymptomatic coeliac disease in T1DM patients. Does screening and treatment

by a GFD outweigh the harms of managing a population already burdened with an established chronic illness? The benefit of an economically and socially difficult GFD in asymptomatic screen-detected CD patients remains controversial[19,20]. It was observed that asymptomatic patients with CD reported better self perceived health and less concern with their disease prior to dietary modification[21]. The CD-DIET Study (Coeliac Disease and Diabetes - Dietary Intervention and Evaluation Trial) is a multicentre randomized controlled trial in Canada aimed at evaluating the safety and efficacy of a GFD in patients with asymptomatic coeliac disease and T1D over 1 year[22]. This study will add important data in the discussion about screening for CD in T1DM patients. Furthermore, prospective longitudinal studies are needed to establish a screening interval for CD in T1DM patients.

A recent systematic review of 9 longitudinal cohort studies involving 11 157 children and adolescents with 587 cases of biopsy-proven CD found that 79% of patients with coeliac disease and T1DM were diagnosed with coeliac disease within 5 years of T1DM diagnosis[23]. Therefore, they recommend no screening after 5 years of T1DM diagnosis, however, longitudinal studies investigating this, especially in adults, are lacking. Another question with regards to screening for CD in T1DM patients is how to detect overt CD as a recent study has shown that TG2A levels decrease in about 40% of children with T1DM[24]. Finally, studies are needed which investigate whether testing for CD in T1DM patients is cost-effective.

Table 1: CD as a candidate for screening in T1DM patients[25-29].

WHO criteria	
That the disease is common and well defined	CD occurs in approximately 6% of the patients with T1DM (25)
Screening tests are simple, safe and accurate	TG2A screening offers high sensitivity and specificity (26)
The screening test should be culturally acceptable	Screening seems to be culturally accepted in most parts of the world
Treatment is available	GFD offers symptomatic relief and will lead to mucosal healing (27)
Clinical detection is difficult	The clinical picture of CD varies and a delay in diagnos is frequent (28)
If undiagnosed and untreated the disease will lead to severe complications	Symptomatic patients will develop complications (29) Studies in screen detected, asymptomatic, CD in T1DM patients with long term follow-up are lacking
Testing and treatment is cost effective	No studies in T1DM patients are performed investigating this item

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Nederlandse samenvatting (Dutch Summary)

Samenvatting

Coeliakie is een auto-immuunziekte (een overdreven reactie van het lichaam door de vorming van antistoffen gericht tegen lichaamseigen weefsels) waardoor beschadiging van het slijmvlies van de dunne darm ontstaat door het eten van gluten. De functie van de dunne darm is om voedingsstoffen op te nemen, die belangrijk zijn voor het goed functioneren van het lichaam. Coeliakie resulteert uiteindelijk in malabsorptie, het onvermogen om voedingsmiddelen op te nemen, waardoor ernstige tekorten (bijvoorbeeld van diverse vitamines) kunnen ontstaan[1]. Coeliakie komt vooral voor bij mensen met een genetische predispositie (erfelijke aanleg) voor deze aandoening. Gluten (van het Latijnse *gluten*, lijm) is de naam van een groep eiwitten die wordt aangetroffen in sommige granen (tarwe, rogge, haver, gerst en spelt) en de producten die hiervan worden gemaakt. De behandeling van coeliakie bestaat uit een dieet vrij van gluten. Coeliakie kan een grote verscheidenheid aan klachten veroorzaken, zoals diarree, buikpijn, gewichtsverlies, botontkalking en bloedarmoede[1].

Type 1 diabetes mellitus (T1DM) is een auto-immuunziekte waarbij het eigen afweersysteem de cellen die insuline in de alvleesklier produceren aantast. Een van de belangrijkste gevolgen van een tekort aan insuline is dat de bloedsuikers (glucose) niet goed kunnen worden opgenomen in de cellen van het lichaam en hierdoor tot hoge waarden kan stijgen. Klassieke symptomen van T1DM patiënten bestaan uit polydipsie (veel dorst), polyurie (veel urine productie), vermoeidheid en uiteindelijk, indien zonder behandeling, coma als gevolg van een ketoacidose (verzuring). Patiënten met T1DM moeten constant hun bloedglucosespiegel in de gaten houden en vaak meerdere malen per dag insuline injecties toedienen[2]. Wanneer de bloedglucosespiegel minder goed gereguleerd is, ontstaat op de lange termijn het risico op ernstige complicaties zoals schade aan het netvlies, nieren en zenuwen.

Dat deze twee auto-immuunziekten (coeliakie en type 1 diabetes mellitus) vaak samen voorkomen is sinds 1970 bekend. De prevalentie van coeliakie in de algemene bevolking is ongeveer 1% terwijl dit bij patiënten met T1DM ongeveer 6% is[3,4]. Factoren die een rol spelen bij deze verhoogde prevalentie zijn genetische-, immunologische- en omgevingsfactoren[5,6]. Beide aandoeningen hebben klinische gevolgen voor de patiënt op de lange termijn. Wat de gevolgen voor de patiënt zijn indien beide aandoeningen aanwezig zijn, is grotendeels onbekend.

In dit proefschrift worden verscheidene klinische en genetische aspecten van patiënten met coeliakie en T1DM onderzocht. Het proefschrift wordt besloten met de vraag of screening naar coeliakie nodig is bij volwassenen met T1DM.

In **hoofdstuk 2** hebben we onderzocht hoe de huidige klinische praktijk is aangaande het diagnosticeren van coeliakie bij patiënten met T1DM. We onderzochten een Nederlandse groep van 118 patiënten met zowel coeliakie als T1DM. In deze retrospectieve studie verzamelden we gegevens over de startleeftijd van T1DM, leeftijd van coeliakie diagnose, aard van de coeliakie-gerelateerde klachten, duur van de klachten voorafgaand aan de coeliakie diagnose, familie anamnese, comorbiditeit en HLA-DQ type. Het HLA-DQ type codeert voor eiwitten die bij de immuunrespons betrokken zijn, een bepaald HLA-DQ type (HLA-DQ 2.5 en DQ 8) geeft een verhoogd risico op de ontwikkeling van coeliakie. We observeerden een bimodale distributie van de leeftijd waarop coeliakie bij patiënten met T1DM werd gediagnosticeerd. Er was een piek van de coeliakie diagnose rond de leeftijd van 10 en 45 jaar. Verder vonden we dat een hoog percentage van deze groep patiënten langdurig klachten had (meer dan 5 jaar) die later aan coeliakie toegeschreven konden worden. Deze observatie impliceert dat behandelaren van patiënten met T1DM eerder aan coeliakie dienen te denken en hierop dienen te screenen.

Dat (niet-gediagnosticeerde) coeliakie leidt tot klachten en/of complicaties als gewichtsverlies, buikpijn, diarree en osteoporose is bekend maar wat de gevolgen van coeliakie zijn bij patiënten met T1DM is grotendeels onbekend. In **hoofdstuk 3** hebben we onderzocht wat de gevolgen van coeliakie zijn op het ziekte beloop van volwassenen met T1DM. Hierbij werd het effect van coeliakie onderzocht op de glucoseregulatie en op diabetische complicaties als retino- en nefropathie. We vergeleken 31 patiënten met zowel T1DM als coeliakie met 46 patiënten met alleen T1DM waarbij er was gematched voor leeftijd, geslacht, duur van de T1DM en HbA1c levels. HbA1c is een maat voor de regulatie van de bloedsuikerspiegel over 6 tot 10 weken. We observeerden dat coeliakie geen invloed had op de HbA1c waarde voor, tijdens en na de diagnose van coeliakie. Verder vonden we dat coeliakie patiënten met een glutenvrij dieet en T1DM minder vaak retinopathie (beschadiging van het netvlies) hadden dan T1DM patiënten zonder coeliakie.

Onze hypothese voor de lagere prevalentie van retinopathie bij patiënten met coeliakie en T1DM was dat deze patiënten lagere spiegels van Advanced Glycation End products (AGE's) zouden hebben[7] hetgeen is onderzocht in **hoofdstuk 4**. AGE's zijn overdreven versuikerde eiwitten, die beschadigd zijn door de zogenaamde Maillard reactie. AGE's kunnen fysiologisch gevormd worden bij normaal metabolisme (stofwisseling) en veroudering maar in situaties zoals bij diabetes mellitus kan door hoge bloedglucosewaarden de AGE concentratie hoger dan normaal worden. Samen met AGE receptoren dragen AGE's bij aan de ontwikkeling van atherosclerose (aderverkalking)[8,9]. Naast deze zogenaamde endogene productie van AGE's kunnen deze ook stijgen door inname van voedsel dat AGE's bevat[10]. Een Australische studie liet zien dat kinderen met T1DM en coeliakie lagere AGE levels hebben waarbij werd gesuggereerd dat een glutenvrij dieet minder exogene AGE's (lees: minder inname van AGE's via de voeding) bevat en er dus minder vasculaire schade zal ontstaan[7]. In **hoofdstuk 4** werd onderzocht of er verschillen zijn in AGE levels bij 25 volwassen patiënten met zowel T1DM als coeliakie versus 25 patiënten met alleen T1DM versus 25 gezonde controles. AGE concentraties werden in de huid gemeten door middel van de AGE reader ® en in het serum werd een receptor van AGE (RAGE) bepaald. Er werden geen verschillen gevonden in concentratie van de AGE's gemeten in de huid of in serum RAGE levels tussen T1DM patiënten met of zonder coeliakie. De AGE levels waren significant hoger in T1DM patiënten dan in gezonde controles. Deze studie suggereert dat AGE's niet de oorzaak zijn van een verschil in microvasculaire complicaties tussen T1DM + coeliakie en T1DM patiënten.

De behandeling van coeliakie bestaat uit een glutenvrij dieet waardoor de kwaliteit van leven negatief beïnvloed kan worden. In **hoofdstuk 5** werd de kwaliteit van leven onderzocht bij patiënten met coeliakie en T1DM. We vergeleken 57 volwassenen met T1DM en coeliakie met 57 voor leeftijd, geslacht en socio-economische status gematchte patiënten met alleen T1DM en een gezonde controle groep. In de groep patiënten met T1DM en coeliakie hadden vrouwen ten opzichte van mannen een lagere kwaliteit van leven op het gebied van sociaal functioneren, vitaliteit en mentale gezondheid. Verder vonden we dat patiënten met T1DM + coeliakie versus T1DM patiënten zich meer zorgen maken over sociaal functioneren en lange termijn complicaties van T1DM. Een vergelijking tussen patiënten met T1DM + coeliakie en een gezonde controle populatie liet zien dat de eerste groep vooral lager scoort op de subscores gezondheidsperceptie en sociaal functioneren. De conclusie is dat coeliakie een additief negatief effect heeft op de kwaliteit van leven bij patiënten

met T1DM. In de begeleiding, follow-up en behandeling van deze patiënten dient hier aandacht aan besteed te worden.

In **hoofdstuk 6** is gekeken naar genetische verschillen tussen patiënten met coeliakie + T1DM versus patiënten met alleen coeliakie of alleen T1DM. Er werd onderzocht of Single Nucleotide Polymorphisms (SNP´s, variaties in het DNA) die geassocieerd zijn met coeliakie (n=28) of T1DM (n=42) vaker voorkwamen bij patienten met coeliakie + T1DM. Er vond een vergelijking plaats tussen 543 patiënten met T1DM + coeliakie versus 2472 patiënten met alleen T1DM en 2223 patiënten met alleen coeliakie. Er werden twee associatie analyses verricht: dubbele auto-immuniteit versus T1DM en dubbele auto-immuniteit versus coeliakie. De CTLA4 en IL2RA loci waren meer geassocieerd met dubbele auto-immuniteit dan met coeliakie of T1DM alleen. Het HLA-DQ 2.5 haplotype was significant geassocieerd met dubbele auto-immuniteit vergeleken met T1DM alleen (OR 1.44, $P = 0.0003$). In de klinische praktijk is HLA-DQ typering alleen zinvol om de ontwikkeling van coeliakie onwaarschijnlijk te maken in het kader van een T1DM patiënt die HLA-DQ 2.5 en DQ 8 negatief is[6]. Als toevoeging liet een Nederlandse studie zien dat HLA-DQ typering in T1DM patiënten niet onderscheidend is en ook niet kosteneffectief bij screenen op coeliakie[11]. Onze bevindingen suggereren dat het genetische risico voornamelijk is gebaseerd op specifieke allelen en genotypes uit de HLA II regio. Deze informatie kan helpen in het bouwen van genetische risico modellen voor patiënten met coeliakie of T1DM die een risico hebben op de ontwikkeling van dubbele auto-immuniteit.

In het laatste hoofdstuk van dit proefschrift (**hoofdstuk 7**) geven we een overzicht van studies naar het effect van coeliakie op T1DM en bediscussiëren we de vraag of screening gerechtvaardigd is bij volwassenen met T1DM. Onze conclusie is dat de diagnose van coeliakie vaak te laat wordt gesteld en dat de diagnose coeliakie klinische gevolgen heeft voor patiënten met T1DM, namelijk verlaagde botdichtheid, meer microvasculaire complicaties, afname van kwaliteit van leven en een hogere mortaliteit. Ons screenings algoritme adviseert om elke 5 jaar te screenen op coeliakie bij patiënten met T1DM middels antistoffen tegen tissue transglutaminase (TG2A). Indien TG2A positief is, is een duodenumbiopt vereist om vlokatrofie aan te tonen. Indien het duodenumbiopt vlokatrofie aantoont dient een 'behandeling' voor coeliakie te volgen bestaande uit; verwijzing naar diëtiste en starten met een glutenvrij dieet, controle van vitamines + ijzerstatus en een DEXA scan (beoordeling

van de botdichtheid). Indien antistoffen tegen TG2A negatief zijn en de patiënt asymptomatisch is, zou de serologie over 5 jaar herhaald moeten worden. Indien de patiënt wel klachten heeft maar negatieve TG2A dient een andere diagnose overwogen te worden en TG2A na 6 maanden herhaald te worden. Een vroegere diagnose zal mogelijk leiden tot het voorkomen van complicaties en een betere kwaliteit van leven.

Toekomst perspectief

Hoewel in dit proefschrift nieuwe aspecten met betrekking tot klinische en genetische aspecten van patiënten met T1DM en coeliakie zijn weergegeven, blijft verder onderzoek noodzakelijk

Ten eerste, er zijn geen effectieve strategieën om autoimmuunaandoeningen te voorkomen of genezen. Coeliakie en T1DM hebben beide een sterke associatie met HLA, hetgeen betrokkenheid van het adaptieve immuunsysteem weergeeft en de aanwezigheid van antistoffen is karakteristiek. Bij de patiënt met coeliakie zijn er 4 componenten die interactie hebben; gluten, TG2A, HLA-DQ2/8 en T cellen[12]. Echter, er is weinig informatie over omgevingsfactoren die dit auto-immuunproces in gang zetten. Bij coeliakie is de associatie met het HLA-DQ type erg sterk; ongeveer 95% van de patiënten is HLA-DQ2 positief en de andere zijn HLA-DQ8 positief[13]. Desondanks heeft 40% van de algemene populatie ook dit HLA-DQ type maar is het maar 1% van de algemene bevolking die coeliakie ontwikkeld. Dit suggereert dat er een omgevingsfactor aanwezig is die dit veroorzaakt die tot op heden onbekend is.
Veranderingen in de samenstelling van de darmflora spelen mogelijk een rol bij de ontwikkeling van coeliakie[14]. Studies observeren een verschillende microbiota bij coeliakiepatiënten met of zonder een glutenvrij dieet, vergeleken met gezonde personen[15,16]. Om beter te begrijpen of veranderingen in de darmflora een oorzaak of gevolg zijn van coeliakie zijn prospectieve studies nodig bij gezonde kinderen die het risico hebben om coeliakie te ontwikkelen[17].Verschillende studies hebben gekeken naar verschillende omgevingsfactoren voor de ontwikkeling van T1DM (virale infecties, geen borstvoeding, consumptie van granen) echter geen van deze factoren is sterk geassocieerd met T1DM. Darmflora kan een rol spelen aangezien een recente studie in mensen liet zien dat bij kinderen met T1DM er een minder divers en minder stabiele darmflora is in vergelijking met gezonde kinderen[18]. Meer prospectieve studies zijn nodig om het microbioom als omgevingsfactor voor T1DM te onderzoeken.

Ten tweede, screening op coeliakie voldoet aan bijna alle WHO criteria voor screening (tabel 1). Een belangrijke vraag is of T1DM patiënten met asymptomatische coeliakie behandeld moeten worden voor coeliakie. Het is maar de vraag of een duur en sociaal invaliderend dieet zoals het glutenvrij dieet voor asymptomatische coeliakie patiënten nodig is[19,20]. Een eerdere studie liet zien dat asymptomatische coeliakie patiënten een betere kwaliteit van leven hadden voordat ze met een glutenvrij dieet moesten starten[21]. De CD-DIET Study (Coeliac Disease and Diabetes - Dietary Intervention and Evaluation Trial) is een multi centra gecontroleerde gerandomiseerde trial in Canada waarbij de veiligheid en effectiviteit van een glutenvrij dieet in een T1DM populatie met asymptomatische coeliakie onderzocht gaat worden gedurende 1 jaar[22]. Deze studie zal belangrijke gegevens toevoegen in de discussie omtrent screening op coeliakie in T1DM patiënten. Bovendien, prospectieve longitudinale studies zijn nodig om een eventueel screeningsinterval te kunnen bepalen.

Een recente systematische review onderzocht 9 longitudinale cohort studies om te beoordelen of screening naar CD bij T1DM geïndiceerd is[23]. Zij includeerden 11157 kinderen + volwassenen met T1DM waarvan er 587 biopt bewezen coeliakie hadden[23]. Bij 79% van de patiënten met T1DM en coeliakie werd coeliakie binnen 5 jaar na T1DM diagnose gesteld. Daarom suggereren zij dat er 5 jaar na T1DM diagnose niet meer gescreend hoeft te worden op coeliakie. Echter, longitudinale studies die dit prospectief onderzoeken, met name bij volwassenen, ontbreken. Een andere vraag aangaande screening is hoe coeliakie het best kan worden gedetecteerd in T1DM patiënten. Een recente studie liet zien dat TG2A levels dalen bij 40% van de kinderen met T1DM[24]. Ten slotte, studies die de kosteneffectiviteit van screening op coeliakie bij T1DM onderzoeken zijn nodig.

Tabel 1: Coeliakie als een kandidaat voor screening in T1DM patienten[25-29].

WHO criteria	
Dat de ziekte frequent voorkomt en goed gedefinieerd is	De prevalentie van coeliakie bij T1DM patienten is 6% (25)
Screening test is simpel, veilig en accuraat	TG2A hebben een hoge sensitiviteit en specificiteit (26)
Screening wordt geaccepteerd	Screening lijkt in de meeste gedeelte van de wereld geaccepteerd te worden
Behandeling is mogelijk	Glutenvrij dieet is de behandeling en leidt tot herstel van de darmmucosa
Klinische vaststelling is moeilijk	Het klinische spectrum van coeliakie varieert en de diagnose coeliakie wordt vaak te laat gesteld (28)
Indien niet gediagnosticeerd en onbehandeld leidt coeliakie tot complicaties	Symptomatische patienten ontwikkelen complicaties (29) Studies bij asymptomatische coeliakie patienten met T1DM ontbreken
Testen en behandelen is kosteneffectief	Studies bij patienten met coeliakie en T1DM ontbreken

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About the author

Curriculum Vitae

Sjoerd Feitze Bakker werd geboren op 27 januari 1984 te Rotterdam. Op jonge leeftijd verhuisde hij met zijn gezin naar Best in Brabant. Hij volgde zijn Voorbereidend Wetenschappelijk Onderwijs (VWO) aan het Jacob Roelands Lyceum te Boxtel. Vervolgens behaalde hij zijn propedeuse van de studie Biomedische Wetenschappen aan de Vrije Universiteit Amsterdam. Hierna werd in 2003 gestart met de studie Geneeskunde aan de Vrije Universiteit Amsterdam. Gedurende zijn studie volgde hij (wetenschappelijke) stages in The Apostolic Hospital te Banga Bakundu (Kameroen), Kalafong Hospital te Johannesburg (Zuid-Afrika), Queensland Institute of Medical Research te Brisbane (Australië) en Queen Elizabeth Hospital te Birmingham (Verenigd Koninkrijk). Door een bijbaan als onderzoeker bij dr. A.A. van Bodegraven op de afdeling Maag- Darm- Leverziekten van het VU Medisch Centrum werd zijn interesse in de gastroenterologie gewekt. In 2010 behaalde hij zijn artsenbul en hierna begon hij met promotieonderzoek onder leiding van prof. dr. C.J.J. Mulder bij de afdeling Maag- Darm- en Leverziekten van het VU Medisch Centrum. De vooropleiding Interne Geneeskunde werd vanaf 2013 tot en met 2015 gevolgd in het Medisch Centrum Alkmaar (opleider dr. F. Stam). In hetzelfde ziekenhuis vervolgde hij zijn opleiding tot maag-darm-leverarts (opleider dr. M. Klemt Kropp). Vanaf mei 2016 is hij werkzaam als maag-darm-leverarts in opleiding in het VU Medisch Centrum te Amsterdam (opleider dr. M.A.J.M. Jacobs) en begin 2019 zal hij zijn opleiding afronden.

List of Publications

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